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REINSTATEMENT OF BIOLOGICAL ACTIVITY IN SEVERELY DISTURBED SOILS: EFFECTS OF MINING ON THE MICROBIOLOGY OF THREE MINESPOILS AND THE MICROBIAL DEVELOPMENT IN THE MINESPOILS AFTER AMENDATION AND PLANTING

by

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EXECUTIVE SUMMARY

Soil microorganisms and their activities are the major vectors in the decomposition of plant litter and the subsequent transformation and flow of such essential plant nutrients as nitrogen and phosphorus. The end result of their activities is the stabilization of soil organic matter upon which the development of a self-sustaining vegetative cover is based. Consequently, a project was initiated with the following objectives in mind:

1. to determine the immediate effects of coal and bitumen mining on a variety of soil microbiological factors.
2. to provide detailed information on the rates of redevelopment of biological activity when various organic and inorganic amendments are applied singly to various minespoils and subsequently planted with different herbaceous and woody plant species.

The effects of surface mining on soil microbial populations, microbial activity and decay potential were studied in a prairie grassland, a subalpine spruce-fir forest and a jack pine forest (oil sands) in Alberta. In general, mining drastically changed the composition of the bacterial and fungal communities and caused significant reductions in bacterial, actinomycete numbers, lengths of fungal hyphae, microbial respiration, microbial biomass C and ATP. However, the decomposition of cellulose filter paper placed in the field for two to three years was more rapid in the mined than unmined sites. The decrease in soil microbial activity after mining was attributed to the loss of organic matter since microbial biomass C and soil organic matter in revegetated subalpine minesoils were observed to be highly correlated.

Procedures for improving the microbial status of the prairie grassland and subalpine coal minespoils and the oil sands tailings were studied by treating each one with three different organic or inorganic amendments and then planting each with four different plant species. The grassland spoil was treated with topsoil, anaerobically-digested sewage sludge, gypsum or left untreated and planted with fall rye, crested wheatgrass, Russian wild-rye or rambler alfalfa. The subalpine minespoil and oil sands tailings received fibrous peat, sewage sludge, mineral fertilizer or no amendment. The plant species tested on the subalpine spoil included slender wheatgrass, white spruce, alsike clover and laurel leaf willow while those on the tailings sand were slender wheatgrass, sainfoin, jack pine and bearberry. Individual plant species were studied on each amendment treatment. The response of mixtures of plant species or combinations of amendments were not examined. The effects of amendation and plant growth (fall rye on the grassland minespoil; slender wheatgrass and white spruce on the subalpine spoil; and slender wheatgrass and jack pine on the tailings sand) on soil microbial development were monitored over three to four years.

Application of sewage sludge or topsoil to the grassland spoil was highly effective in increasing microbial activity and biomass C, particularly in the upper 5 cm of the amended spoil. The development of microbial activity and biomass appeared to be linked to primary production since both parameters increased as shoot production by fall rye increased. The increased microbial biomass maintained itself for at least one year after fall rye failed, but its metabolic activity was reduced to pre-planting levels. The decay of filter papers appeared to be related to density of plant cover rather than amendment type with decomposition in the topsoil and sewage sludge treatments being faster than that in the gypsum and control treatments. No significant treatment effects on filter paper decay were measured after growth by fall rye ceased, while the decay potential in the topsoil and sludge treated spoil increased as plant cover by rambler alfalfa increased. The N_2 fixation potential of rambler alfalfa was not significantly affected by amendation, although measurements were highly variable.

The microbial status of the subalpine and oil sands spoils was most improved by the addition of peat while treatment with sewage sludge or fertilizer was less effective. The effects of amendation and planting were restricted mainly to the top 5 cm of the treated spoils. Although CO_2 efflux from the unplanted, peat amended minespoils increased substantially over the four year term of the study,

loss on ignition estimates indicated no significant loss of stable organic matter from this treatment. The fast growing, highly productive slender wheatgrass was more effective in stimulating the development of microbial activity and biomass C in the variously treated minespoils than the slow growing white spruce or jack pine were, again suggesting that plant productivity and soil microbial productivity are closely related. Rates of microbial development appeared to be dependent on the input of root exudates, sloughed root material and dead roots and shoots from the primary producers, which, in the case of slender wheatgrass, was particularly high in the sewage sludge treatment.

During the first two years after planting, cellulose filter paper placed in the subalpine spoil decomposed most rapidly in the sewage sludge treatment regardless of vegetation type, while those placed in the oil sands minespoil decayed fastest in the sewage sludge and fertilizer treatments planted with slender wheatgrass. Over the long term (four years), cellulose decay potential of the amended and planted subalpine spoil was not significantly altered, but the decay potential of the sludge treated sand planted with slender wheatgrass was considerably reduced. In both minespoils, the short term decomposition (one year) of slender wheatgrass leaves was faster than that of stems. Alsike clover leaves decayed more rapidly than slender wheatgrass leaves in the subalpine minespoil whilst sainfoin leaves decayed more slowly than slender wheatgrass leaves in the oil sand minespoil. Neither grass nor legume litter decomposition was significantly affected by amendation. The decay rates of filter paper could not be extrapolated to predict decay rates of plant litter. The N_2 fixation capacity of alsike clover was not significantly influenced by amendation, but sewage sludge inhibited N_2 fixation by sainfoin after the third growing season.

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1. INTRODUCTION

The chemical, physical and biological properties of a minespoil are considered to be the main factors influencing the successful establishment of a vegetative cover (Vogel, 1981). Although the chemical and physical aspects of minespoils and their influence on plant success have received a great deal of attention, the biological properties of minespoils have, until recently, been largely ignored. Both Cundell (1977) and Jurgensen (1978) stressed the importance of soil microorganisms to vegetation establishment and discussed the potentially important roles microbial processes could have in soil formation on minespoils.

The measurement of microbial activity and biomass in minespoils is considered important for the following reasons:

i) microbial activity is one of the major factors responsible for the decomposition of plant litter and roots resulting in both the stabilization of soil organic matter and the release of nutrients for subsequent plant growth.

ii) soil microorganisms behave as both sources and sinks of essential plant nutrients and are instrumental in the transformation and flow of C, N, and P (Ausmus et al., 1976; Paul and Voroney, 1980); also, because of their relatively rapid growth rate, they are potentially capable of quickly immobilizing nutrients which would otherwise be lost through leaching.

iii) soil microorganisms are metabolically highly diverse and therefore have the capability to adapt to the low nutrient levels and adverse chemical and physical conditions often found in minespoils (Parkinson, 1978).

iv) symbiotic microorganisms such as N_2 fixing bacteria and mycorrhizal fungi are considered crucial in spoils where both N and P can occur at such low levels that plant growth is adversely affected.

Research regarding microorganisms and the reclamation of minespoils has, to date, dealt mainly with the effects of mining disturbance on microbial population parameters (i.e. numbers and types of bacteria, actinomycetes and fungi), physiological groups of microorganisms, general microbial activity, decomposition and nitrogen mineralization processes, and mycorrhizal inoculum potential. Many of these studies have included data on the microbiological characteristics of minespoils after applying amendments and/or planting.

Until a decade ago, much of the research dealing with the microbiological properties of minespoils had been carried out by Wilson and his co-workers on coal strip-mine spoils in the eastern U.S. Much of this work was summarized in Wilson (1965). One of the observations made in his investigations was that the total numbers of bacteria, fungi and actinomycetes were generally lower in unvegetated spoil compared with the revegetated spoil and nearby undisturbed areas. The root region (root surface and rhizosphere) was particularly effective in stimulating numbers of bacteria. Müller (1973) also recorded lower numbers of bacteria and actinomycetes in fresh brown-coal minespoils in Poland than in revegetated minespoils and, like Wilson (1965), concluded that microbiological activity improved as revegetation progressed. Both Müller (1973) and Wilson (1965) observed that quantitative assessments of fungi were not dramatically influenced by the presence of vegetation. However, these data must be viewed with caution because the dilution plate technique was used in both these studies - a technique which has been shown to select for fungal spores rather than the active hyphae (Warcup, 1955). More recently, Fresquez and Lindemann (1982) observed from data obtained using soil dilution plating that the undisturbed soil and soil from a reclaimed area in New Mexico, had similar bacterial, actinomycete and fungal numbers, but that microbial numbers in the nonvegetated spoil were low. However, when the spoils were amended with alfalfa or sewage sludge microbial numbers increased - a result attributed to the addition of a microbiologically available carbon source. Like Wilson (1965), Fresquez and Lindemann (1982) also recorded a stimulation of microbial numbers in the rhizosphere soil particularly in the presence of highly organic amendments.

Studies on the types of bacteria and fungi found in minespoils are limited. Müller (1973) listed the predominant fungi and bacteria isolated from minespoils in Poland and observed that no major differences existed between genera of micro-organisms in bare spoil and spoils revegetated for 2-6 yrs. Data collected by Wilson (1965) also inferred that the same types of fungi occurred in a nonvegetated spoil as in a vegetated spoil. In contrast, Lawrey (1977) and Fresquez and Lindemann (1982) reported that there were generally fewer fungal genera in minespoil than in undisturbed soil. The addition of organic amendments increased the diversity of the fungal isolates (Fresquez and Lindemann, 1982). No attempts were made to estimate the frequencies of occurrence of the fungi isolated in these studies.

The transformation of nitrogen in organic matter to ammonium and finally to nitrate is mainly a bacterial process. Since nitrogen is an essential plant nutrient and is generally lacking in mined soils, many researchers have concentrated their investigations on the effects of mining on the organisms involved in the ammonifying and nitrifying processes. Wilson (1965), Müller (1973) and Williams and Cooper (1976), all working with acidic minespoils, found that if the pH of the spoil was raised through liming and fertilization, an increase in the numbers of ammonifiers and nitrifiers resulted. Highly acidic minespoils - a consequence of the oxidation of sulphur-bearing pyrites in the spoil by Thiobacillus spp. resulting in H_2SO_4 production - therefore require the application of lime if the nitrification process is to become established. In contrast to the acidic minespoils located in the eastern U.S., Fresquez and Lindemann (1982) working with the neutral to alkaline spoils of the western U.S., stimulated ammonium oxidizers by amending the spoil with alfalfa hay and fertilizer. Although the availability of NO_3-N through nitrification is regarded as necessary to the successful establishment of a vegetative cover on minespoils, Williams and Cooper (1976) have cautioned that a too rapid nitrification of applied ammoniacal fertilizers could result in N losses through leaching, particularly in sandy soils. In fact, Lodhi

(1979) has provided evidence which suggests that as plant succession proceeds on minespoils, the vegetation inhibits the nitrification process thereby conserving N which could otherwise be lost through leaching.

Microbial activity measurements such as soil respiration ($O_2\downarrow$ and $CO_2\uparrow$), adenosine triphosphate (ATP) levels and soil enzyme (phosphatase, dehydrogenase, pectinolyase, urease, amylase) activities have been the parameters must commonly used for determining the effects of mining on microorganisms and the development of a microbial system in minespoils. In general, the results obtained from these measurements have supported the plate count data i.e. disturbance by mining results in a decrease in microbial activity, and the addition of organic matter and the establishment of vegetation leads to an increase in microbial activity. Lawrey (1977) attributed the reduced soil respiration he measured in acidic minespoils to low pH, high levels of Fe and Mn rendered more soluble by the soil acidity and low macronutrient levels. In an earlier study, Hedrick and Wilson (1956) noted that the addition of nitrogen to acidic minespoils stimulated CO_2 production and that raising the spoil pH with calcium hydroxide did not influence CO_2 efflux to the same extent. Respiration was most stimulated by the addition of a complete fertilizer, organic matter (straw) and calcium hydroxide. Recently, Stroo and Jencks (1982) compared microbial respiration and enzyme activities in undisturbed soils and in minespoils at various stages of development. As expected microbial activity was generally reduced by mining, but as organic matter and nitrogen accumulated through revegetation, activity increased. Respiration, amylase and phosphatase were significantly correlated and related to organic C and N levels. The low levels of phosphatase activity in the minespoil led the authors to suggest that P cycling in the spoil could be impaired. Soil enzyme assays (phosphatase, pectinolyase, dehydrogenase) have also been used to characterize the microbial components in neutral to alkaline coal strip mine spoils in the western U.S. (Hersman and Temple, 1979; Fresquez and Lindemann, 1982). Soil ATP levels have been shown to be greater in native grassland soils than minespoils in Montana (Hersman and Temple, 1978) and have demonstrated a high correlation with respiratory ($CO_2\uparrow$) activity in a variety of minespoils (Hersman and Temple, 1979). Measurements of ATP, dehydrogenase, phosphatase and microbial respiration have also been used extensively for studying the effects of retorted oil shale materials on microbial activity in overlying surface soils (Hersman and Klein, 1979; Sorensen et al., 1981). The results obtained indicated that microbial activity was reduced in soils overlying processed oil shale and that a capillary barrier between the surface soil and the shale would possibly remedy the decreased microbial activity (Sorenson et al., 1981)

As mentioned previously, it is microbial activity which is responsible for much of the decomposition of incoming plant material. On minespoils, the decay process would be particularly crucial since it would be closely related to nutrient availability and the rate of soil genesis. Therefore, it seems surprising that although microbial activity in minespoils has been studied extensively, the rate of decomposition of incoming plant litter and roots produced by the the evolving plant community has received limited attention. On acid minespoils in Ohio, it was observed that strip mining had inhibitory effects during the early stages of decay, but these effects were overcome over the long term (Lawrey, 1976). The slow initial decomposition on the mine spoil was believed to be the result of low soil pH, high levels of trace metals, and a decrease in fungal diversity and soil respiration. In a subsequent study, Lawrey (1977) determined that the decay rate of a variety of litter types (placed in four areas which had been mined and subsequently revegetated) was correlated with soluble carbohydrate, ash and macronutrient components of the litter but decay rates did not differ amongst the four study sites. When investigating decomposition of tall fescue on minespoils in Missouri, Carrel et al. (1979) found that loss of dry weight was more rapid on vegetated minespoil than on bare minespoil. They also noted that litter decomposition in the vegetated minespoil was highly variable and that the initial C/N ratio of the litter was a major factor in determining the rate of decay. Interestingly, loss of nitrogen did not differ between the bare and vegetated minespoil. Lanning and Williams (1979) also reported a relationship (negative) between C/N ratio of grass and clover shoots and roots and their decomposition rates in china clay sand wastes in Britain. Clover shoots and roots were observed to decompose faster than grass shoots and roots with net N release occurring from the clover shoots and roots. These data suggest that the use of legumes in revegetating minespoils would lead to a faster turnover of nutrients immobilized in the plant tissue, and an accumulation of organic matter and N (unless the spoil is sandy when potential losses of N through leaching would be enhanced).

Although the value of legumes for revegetating N-deficient minespoils has been generally recognized, very little research has been conducted on the conditions required by legumes to ensure their establishment (and subsequent N-fixing ability) in minespoils. Rather, revegetation efforts have leaned heavily on the use of expensive fertilizers to raise N to levels where plant (mainly grasses) growth would not be inhibited. A study reported by Skeffington and Bradshaw (1980) on N inputs into china clay wastes through leguminous plants, non-leguminous plants, free-living microorganisms and the atmosphere is one of the few to investigate the possibility of using legumes rather than fertilizer for supplying N in mined soil. They concluded that N-fixation rates by non-leguminous plants were so low that atmospheric N input was greater than input from these species. Also, N-fixation by free-living microorganisms was negligible unless an easily available carbon source was provided. However, legumes demonstrated such high N-fixation rates that they could be recommended as a means for accumulating N in this particular mine waste. In relation to the accumulation of N in legume-planted china clay wastes, it should be pointed out that Lanning and Williams (1979) measured very little N-accumulation in the same sandy china clay wastes, unless leaching rates were reduced through the addition of micaceous residues (Lanning and Williams, 1980).

Another important plant-microbial relationship essential to the successful establishment of a maintenance-free vegetative cover on minespoils is that of the endo- and ectomycorrhizal symbiosis. The role of the mycorrhizal fungi in ensuring plant success in nutrient and often moisture-stressed minespoils has been discussed by Zak _et_ _al_. (1984) and Danielson _et_ _al_. (1984).

With the exception of a few studies such as that reported by Stroo and Jencks (1982) it can be seen from this brief survey of the literature that much of the research dealing with the microbiological aspects of minespoils has emphasized the immediate effects of mining on various microbial parameters. Very little attention has been directed towards investigating the development of the microbial system as a vegetative cover becomes established. Also, methods of accelerating the development of a productive soil-plant system on minespoils through the incorporation of various organic and inorganic amendments have not been widely studied. As a result, the research reported was initiated with the following objectives:

1. to obtain information on the immediate effects of mining coal and bitumin on a variety of soil microbiological components (bacterial numbers and types, fungal types, hyphal length, soil respiration, microbial biomass C, asymbiotic N_2 fixing potential) and soil decomposition potential at three different minesites in Alberta.

2. to provide detailed information on the rates of redevelopment of biological activity (particularly soil respiration and microbial biomass C) when organic and inorganic amendments are applied to spoil from each of the three minesites and then planted with various herbaceous and woody plant spp.

3. to study the relationship between soil microbial activity and biomass and the decomposition of a pure cellulose substrate and incoming plant litter in each of the variously amended spoils planted with grass or legumes.

4. to assess the effects of amendation on the nodule formation and N_2 fixing ability of each of the legumes planted in each spoil.

Data regarding the effects of amendation of the three minespoils on selected soil chemical and physical characteristics, plant growth and nutrient levels in the foliage of selected plant species are presented in Visser et al. (1984), whilst Danielson et al. (1984) and Zak et al. (1984) have reported the results obtained on the ectomycorrhizal and vesicular-arbuscular mycorrhizal development of some of the plant species.

The effects of single amendments (not various combinations of amendments) on the soil respiratory activity and microbial biomass C development in the three test spoils, and how these measurements related to plant growth are the main aims of this report.

2. MATERIALS AND METHODS

The three areas chosen for the study included a shortgrass prairie site which had been mined for coal in the early 1950's and subsequently abandoned (Bow City, Alta.); a subalpine boreal forest site which is currently being mined for coal (Luscar, Alta.) and a boreal forest site which is currently being mined for oil (Suncor site, Ft. McMurray, Alta.). Short descriptions of these sites have been presented by Visser et al. (1984).

2.1 FIELD STUDY

2.1.1 Plot Design and Sampling Procedure

At each study site, five sampling plots, each 5 m x 5 m, were staked out within a 15 m x 15 m sampling frame in an area disturbed by mining and also in an adjacent undisturbed area (with the exception of the oil sands site where due to the inaccessibility of the disturbed area, plots were established only in the undisturbed jack pine woodland). Each of the five sampling plots was subsequently divided into 100 subplots, each 0.25 m x 0.25 m. These plots were used for sampling soil for general microbiological studies. Decomposition studies were performed in 5 m x 15 m plots adjacent to those used for the microbiological analysis.

The sampling procedure consisted of randomly choosing one of the subplots in each of the disturbed and undisturbed plots at each of the sites, and removing a soil core (5.5 cm dia.) and a larger 1 to 2 L sample from the middle of the subplot. In the disturbed sites, where there was no horizon development, 0-5 cm and 5-15 cm depths were sampled. In the undisturbed sites, samples were removed to a depth where organic surface and inorganic subsurface soil would be included.

Samples were removed from the following horizons in each of the undisturbed sites: the A_h and B_m in the grassland soil, the H and B_m in the subalpine soil and the FH and B in the oil sand soil. Subsamples from the soil cores were processed for various microbiological parameters while general microbial activity was measured in the larger samples. Another set of soil samples was randomly removed from each of the sites for assessment of asymbiotic N_2 fixation. All sampling was performed in September or October. Samples were placed in plastic bags and stored at 5°C. They were sieved through a 2 mm mesh screen and processed as quickly as possible to avoid possible storage effects on the microbial components. To determine the effects of disturbance on selected soil chemical and physical parameters, subsamples from each depth or horizon were air dried, bulked and tested for sodium adsorption ratio (SAR), organic and CO_3-C, total N, P, Fe, Ca, Mg and Na. Details of the methods used are presented in Visser et al. (1984).

2.1.2 Procedures for the Determination of Microbial Parameters

2.1.2.1 Numbers of bacteria. A soil suspension was prepared by homogenizing 1 g soil in 99 ml sterile, dilute peptone (0.13%) at high speed for 3 min in a Waring blender. Serial dilutions were then prepared and 0.1 ml of the 10^{-5}, 10^{-6}, 10^{-7} dilutions spread onto pre-dried, half-strength PYE (2.5% peptone, 0.5% yeast extract) agar plates. Five replicate plates per dilution per soil sample were plated. Colonies were counted after 7-19 days incubation at 15°C.

2.1.2.2 Types of bacteria. Using a randomized grid method, 12 bacteria per replicate sample were selected and placed in pure culture resulting in 60 isolates per depth per site. The isolates were placed into broad taxonomic groups using the methods outlined by Nelson and Visser (1978).

2.1.2.3 Numbers of actinomycetes. The same homogenate, same serial dilutions and same techniques were used for quantifying the actinomycetes as those used for the bacteria, with the exception that 0.1 ml of 10^{-4}, 10^{-5}, 10^{-6} dilutions were spread onto 3 replicate, pre-dried chitin agar (Hsu and Lockwood, 1975) plates per soil sample. The plates were incubated for at least 14 days at 15°C prior to counting.

2.1.2.4 Length of fungal mycelium. 2 g and 1 g subsamples were removed from the inorganic and organic soil replicates respectively and fixed in Bouin-Hollande fixative until time of analysis. Agar films were then prepared using a modified Jones and Mollison (1948) agar film technique, i.e. soil samples were macerated in water and fixative in a Waring blender for 3 min and from the resulting suspension, soil + water agar films prepared, dried, mounted and observed using phase contrast microscopy. The line intersect method (Olson, 1950) was used to measure hyphal lengths on the films and empty (inactive) hyphae were distinguished from those with cell contents (active) by the criteria described by Frankland (1975). Five films (50 observations/film) were assessed for each soil replicate.

2.1.2.5 Types of fungi. 2.5 g and 5 g soil samples were removed from the organic and inorganic soil replicates respectively. Samples were serially washed (30, 1 min washes with sterile water) using the apparatus and methods described by Bissett and Widden (1972). Excess moisture was removed from the washed soil samples, and soil particles were then plated on 2% malt extract agar containing 100 ppm streptomycin and 50 ppm chlorotetracycline. The size of the particles plated ranged from .2-.5 mm for the organic soils and .5-1 mm for the inorganic soils. Fifteen particles (two/plate) were plated from each of the organic soil replicates and 20 particles (four/plate) were plated from each of the organic soil samples. More particles were plated from the inorganic than organic soils since organic soils are more heavily colonized by fungi than are inorganic soils. The plated soil particles were incubated at 15°C and fungi emerging from each particle isolated and identified.

2.1.2.6 Soil respiration (CO_2 efflux). Each soil sample was sieved through a 2 mm mesh sieve and as many roots as possible were removed from the sample to avoid interference from live root respiration. All roots were removed since the distinction between live and dead roots is rather tenuous. Moisture contents were

determined for each sample and samples were remoistened with distilled water if moisture levels were believed to be limiting to microbial activity. Sieved replicate samples, each equivalent to 100 g dwt, were thoroughly mixed and then placed in plastic tubes, stoppered with plastic foam plugs, and connected to an "Ultragas 3" CO_2 analyzer (Wöstoff Company, Bochum, Germany).
The CO_2 efflux was measured hourly at room temperature (22-23°C) until CO_2 evolution had stabilized. At that point (20-24 hr after beginning CO_2 measurements) respiratory activity was calculated as ml CO_2 $\uparrow$ 100 g^{-1} dwt soil hr^{-1}.

2.1.2.7 Microbial biomass C. The same soil samples as those used for CO_2 efflux measurements were used for estimating microbial biomass C (i.e. the amount of C in the living, non-resting microbial biomass). Microbial biomass C was determined using the technique described by Anderson and Domsch (1978) which is based on the measurement of glucose stimulated respiration prior to the commencement of microbial growth. The relationship between the glucose stimulated CO_2 evolution from a soil sample and the microbial biomass in that sample was determined by Anderson and Domsch (1978) i.e. 1 ml $CO_2 \cdot h^{-1}$ measured at 22°C is equivalent to 40 mg microbial biomass C. Optimal glucose concentrations for maximum respiratory response were determined for representative soil samples from each soil horizon or soil depth and these glucose concentrations were then used to determine the microbial biomass C levels in the replicate samples.

2.1.2.8 Adenosine 5'-triphosphate (ATP) measurements. 1 g subsamples from each soil replicate were extracted with sodium bicarbonate and chloroform and the ATP subsequently measured using the luciferan-luciferase assay and a JRB Model II Photometer to measure the light emitted (Paul and Johnson, 1977).

2.1.2.9 N_2 (C_2H_2) fixation. The acetylene (C_2H_2) reduction assay similar to that of Hardy et al. (1968) but modified by Paul et al. (1973) was applied to each soil sample to determine N_2 fixation potential. Sieved, remoistened soil was placed in standard sized tubes which were then sealed in 909 ml Mason jars. Ten percent of the atmosphere volume in each jar was removed with a syringe and replaced with C_2H_2. Samples were incubated with C_2H_2 for 1 hr at 15°C prior to making C_2H_4 measurements. The following controls were included: empty jar, empty jar containing 0.1 atm C_2H_2 and jar containing a soil core and no acetylene. The rate of ethylene (C_2H_4) production (after subtraction of any C_2H_2 detected in the controls) was used to calculate nitrogenase activity in the soil, using a theoretical ratio of 3 to 1 between C_2H_4 production and N_2 fixed (Hardy et al. 1973).

2.1.2.10 Decomposition of standard substrates. Filter paper (Whatman #1, 5.5 cm dia.) was dried to a constant weight at 35°C, bagged in 1 mm nylon mesh and tagged. The filter paper was left unamended or amended with ammonium nitrate to adjust the C/N ratio of the paper to 25/1. Since filter paper lacks nitrogen and since soil N is often limiting, particularly in soils disturbed by mining, it was hypothesized that the addition of N to the paper could potentially accelerate its decomposition.

To study the effects of disturbance on the decomposition of a recalcitrant substrate, wood (Douglas fir) dowel was also used as a test substrate. The dowel, 2 cm diameter, was cut into 5 cm segments (knots were discarded) dried to a constant wt and tagged. As for the filter paper, wood segments were left unamended or soaked in .36 M ammonium nitrate for 48 hrs prior to drying, weighing and tagging. Blocks of spruce (Picea glauca (Moench) Voss) wood (approx. 3.8 cm x 1.9 cm x 5 cm) were also used as a test substrate but only at the subalpine site.

Fifty replicates of each of the unamended or N-amended filter paper and wood segment treatments were placed on the soil surface at each of the undisturbed and disturbed sites in October. The plots designed for studying the decomposition of the two substrates (one plot for the filter paper and one for the fir dowel at each of the disturbed and undisturbed sites) were 5 m wide x 15 m long and contained 13 rows of 4 stakes placed 1 m apart. The substrates were tied to the stakes (one amended and one unamended filter paper or wood segment/stake) and sampled regularly thereafter. At each sampling time, five filter papers and five wood segments were randomly removed from each of the disturbed and undisturbed sites. Filter papers were cleaned, dried, weighed and ashed, and the ash weight subtracted from the weight remaining prior to ashing. Wood segments were brushed clean, agitated in de-ionized water (to remove adhering soil particles) and dried to a constant weight at 35°C. Results were expressed as % dry weight remaining.

Not all the microbial parameters were investigated at all the sites. A list of the microbial parameters which were studied at each of the experimental sites is presented in Table 1. Since a disturbed plot was not established at the oil sands site, the microbial parameters measured in samples removed from the unamended control plots (pure tailings sand prior to planting) in the tank study (see pp. 6-7 of Visser et al. 1984 for a description of the tank study set-up) were compared with the microbial characteristics of the undisturbed site to assess the effects of disturbance.

2.1.2.11 Decomposition of native litter and wood residues. In addition to following the decomposition rates of standard substrates at each of the disturbed and undisturbed sites, a study was also initiated to examine the rates of decay of native litter and wood debris at the undisturbed sites only. It was felt that this information would be useful in future studies designed to compare rates of decay of plant litter in areas in various stages of reclamation to rates of decay of the same litter in an undisturbed and presumably stable system.

The methods used to examine the decomposition rates of leaf litter and wood at each of the sites were as follows:

1. prairie grassland site - Agropyron sp. standing dead grass litter was clipped approximately 1 cm above the soil surface at the grassland site in late September. Leaf blades which were still

Table 1. Microbial parameters investigated at each of the experimental sites to determine the effects of disturbance.

Parameter	Site and soil horizon or depth (cm)											
	Grassland				Subalpine				Oil Sands			
	Undisturbed		Disturbed		Undisturbed		Disturbed		Undisturbed		Disturbed	
	A_h	B_m	0-5	5-15	H	B_m	0-5	5-15	FH	B	0-5	5-15
Number of bacteria	+	+	+	+	+	+	+	+	+	+	+	+
Types of bacteria	+	+	+	+	+	+	+	+	+	+	+	+
Numbers of actinomycetes	+	+	+	+	+	+	+	+	+	+	+	+
Length of fungal mycelium	+	+	+	+	+	+	+	+				
Types of fungi	+	+	+	+	+	+	+	+	+	+	+	+
CO_2 efflux	+		+		+	+	+	+	+	+	+	+
Microbial biomass C	+		+		+	+	+	+	+	+	+	+
ATP levels	+		+		+	+	+	+	+	+	+	+
N_2 (C_2H_2) fixation	+	+	+	+	+	+	+	+				
Decomposition of standard substrates (filter paper, wood dowel)	+		+		+		+		+		+	

green or yellow at the base (indicating it was the current year's growth) were separated from the other litter, dried to a constant weight at 35°C, weighed into approximately 5 g quantities and bagged in 1 mm nylon mesh bags (size = 13 x 15 cm). Woody branches (approx. 1 cm dia.) of Artemisia cana Pursh (sagebrush) were cut into 5 cm segments, dried to a constant weight at 35°C, weighed and tagged. Fifty litter bags and fifty branch segments were placed in the filter paper and dowel decomposition plots respectively, using the same stakes as those used for the filters and dowel segments. At each sampling time, five replicate litter bags and five branch segments were randomly sampled. Grass blades were removed from the bags and brushed clean if required, while sagebrush wood was given a 30 sec wash in distilled H_2O to remove adhering soil. The % dry weight was then determined for each substrate type.

2. subalpine site - an Abies lasiocarpa (Hook.) Nutt. (alpine fir) tree, blown over by the wind, was located near the margin of the undisturbed sampling site. Green needles and branchwood (2.5-5 cm dia.) were removed from the top 2-4 m (topside branches only) of the tree and used in decomposition studies. Green dying needles and branchwood from one tree only were used in an effort to reduce variation in decay resulting from differences in substrate quality. After drying the needles and branchwood at 35°C, they were treated in the same manner as that described for the prairie grassland Agroypron litter and sagebrush wood. At each sampling time, five replicate needle bags and five wood segments were randomly removed from each plot. The surfaces of the bag were cleaned and needles were then sprayed for 30 sec with tapwater to remove mineral material from the needle surfaces. Wood was treated in the same way as described previously. The % dry wt remaining was then determined for each substrate.

3. oil sands site - needles and branchwood of Pinus banksiana Lamb. (jack pine) were collected from a recently toppled tree as described for the subalpine site. Litter bags and branchwood segments were prepared in the same manner as described previously and placed around the stakes in the filter paper and dowel plots

respectively. Litter bags were placed underneath the lichen mat (i.e. between the sandy soil horizon (A_e) and the lichen) since the lichen would impede the movement of the bag down the soil profile. It was realized that by doing this, decomposition of the needles would be enhanced as a result of better moisture and temperature conditions under the lichen mat. Therefore, 70 pairs of pine needles were strung on each of 15 nylon threads, dried at 35°C, weighed and placed on the surface of the lichen mat. It was hoped that the weight loss of the strung needles would give some indication of the decomposition of needles as they moved through the lichen mat. Unfortunately this method can only be used to monitor weight loss of litter in the early stages of decay since weight loss is overestimated once the litter begins to fragment. Sampling and cleaning of the needles and branchwood was effected in the same manner as that described for the alpine fir substrates.

2.1.3 Effect of Revegetation on Microbial Respiration and Biomass C at the Subalpine Site

Areas which had been revegetated with grasses and legumes for 2, 6 and 7 yr and stockpiled regolith were sampled in June to determine the effects of revegetation on microbial activity and biomass C. These results were then compared to microbial activity and biomass C measured in soil from the undisturbed subalpine forest site (described previously). Five replicate soil samples (1-2 kg each) were removed randomly along a 15 m transect laid out in the revegetated areas and in the undisturbed site. Samples in the revegetated sites were removed from the 0-10 cm depth while those from the undisturbed site were removed from the FH horizon (organic soil) and the B horizon (mineral). Five samples were also randomly taken from the stockpiled regolith (organic and mineral soil and some rock which is removed from above the coal seam and stockpiled for future revegetation of mined areas). Samples were transported to the laboratory in plastic bags and processed as quickly as possible for the following parameters:

1. pH - determined electrometrically using 10 g soil in 20 ml .01 M $CaCl_2$.

2. organic matter content - the Walkley-Black (Nelson and Sommers, 1982) method was used to determine organic matter in soils from the revegetated areas and regolith while loss on ignition (see method in Visser et al. 1984) was measured for soils from the undisturbed site.

3. basal respiration - samples were sieved through 2 mm sieve and CO_2 efflux was then measured using the techniques described previously (i.e. Wöstoff CO_2 analyzer).

4. microbial biomass C - the method of Anderson and Domsch (1978), described previously, was applied to all the soil samples. The optimum levels of glucose required to stimulate maximum microbial biomass were 32,000 $\mu g\ g^{-1}$ undisturbed organic soil (FH layer), 2000 $\mu g\ g^{-1}$ undisturbed mineral (B horizon) soil, 500 $\mu g\ g^{-1}$ regolith soil, 2000 $\mu g\ g^{-1}$ soil revegetated for 2 yr, 2000 $\mu g\ g^{-1}$ soil revegetated for 6 yr and 8000 $\mu g\ g^{-1}$ soil revegetated for 7 yr.

2.1.4 Statistical Analysis of Field Data

Many different statistical techniques were required to analyze the data collected in the field studies. Therefore, for convenience sake, the statistical tests applied to each data set have been footnoted at the end of each table of analyzed data.

2.2 SOIL TANK STUDY

It was decided that the effects of various organic and inorganic amendments on plant growth and soil microbiological characteristics in the three minespoils could be more closely monitored if the research was performed near Calgary, rather than at the more inaccessible field sites. Hence large quantities of minespoil from the grassland, subalpine and oil sand sites were transported to Calgary and each placed into separate plywood frames or soil tanks. In general, each of the three soil tanks was partitioned into 12 plots, 5 m x 7 m each and an approximately 60 cm depth of minespoil placed in each plot. Amendments were then applied to allow three replicate plots per treatment. Anaerobically digested sewage sludge, topsoil and gypsum were applied singly to the grassland minespoil while fibrous peat, sewage sludge and mineral fertilizer were applied to the subalpine minespoil and tailings sand. Three unamended control plots were also set up for each of the three spoils.

Following amendation each plot was subdivided into four subplots with 0.5 m wide walkways between them. In the case of the grassland spoil, each amended subplot was planted with either fall rye, crested wheat, Russian wild rye or rambler alfalfa. The subalpine spoil was planted with slender wheatgrass, alsike clover, and containerized willow or white spruce seedlings, while the amended tailings sand subplots were each planted with slender wheatgrass, sainfoin and containerized bearberry or jack pine. All legume seed had been treated with inoculum of the N_2-fixing bacteria, Rhizobium. Thus this experimental design allowed for three replicates/plant species in each of the four treatments in each spoil. A more detailed description of the experimental design, the amendments and their application, and information regarding the plant species planted in each spoil type have been presented in Visser et al. (1984).

2.2.1 Sampling Procedure

After amendation, but immediately prior to planting, two cores (5.5 cm dia.) and a larger 1-2 L sample were removed from each plot using a simple random sampling procedure. The samples were separated into 0-5 cm deep soil and 5-15 cm deep soil (except in the topsoil and peat amended pots where the 15-25 cm depth - the spoil beneath the amendment - was sampled) as they were placed in plastic bags. The cored soil samples were used for measuring various microbiological parameters and asymbiotic N_2 (C_2H_2) fixation while CO_2 efflux and microbial biomass C were measured in the larger samples.

After planting, samples were randomly removed from the plots in September/October after the first, second and third growing seasons. In the amended grassland spoil, subplots planted with fall rye were sampled. In the subalpine and oil sands spoils, samples were removed from the subplots planted with slender wheatgrass, spruce or jack pine in an attempt to assess the effects of vegetation and vegetation type on the development of microbial activity. Grass plots were sampled by removing a 10.5 cm dia. core (grassland and oil sands spoil) or a 20 cm x 20 cm block of soil (subalpine spoil) from the middle of one randomly chosen quadrat which had previously been clipped for primary production estimates. Samples taken 39 mo after planting slender wheatgrass in the subalpine and oil sands spoil were removed from beneath litterbags containing slender wheatgrass leaf blades to determine whether the microbial activity and biomass in the soil beneath the litter bag was related to the decomposition of the litter. In the spruce and pine plots, samples were extracted from an area between two randomly chosen trees. As for the pre-planting samples, cores or blocks of soils were separated into 0-5 cm and 5-15 cm (15-25 cm in the topsoil and peat-amended plots) deep soil, placed in plastic bags and stored at 5°C until processed. With the exception of the 39 mo slender wheatgrass soil samples (when one sample was removed from beneath each of two litterbags per subplot resulting in two samples per subplot) only one sample was removed from each subplot from each depth at each sampling time.

Although this study was not designed to examine the microbial development in amended but unplanted minespoil, it was decided after the first growing season that it would be useful to sample unplanted soil in an attempt to separate amendment effects on microbial development from the combined plant/amendment effects. Consequently, samples were removed from the pathways surrounding the slender wheatgrass, white spruce and jack pine plots in the subalpine and tailings sand spoils at each of the sampling times. Only pathways between the tank walls and subplots were sampled as these were seldom walked upon. Samples were removed randomly and separated into the two depths in a similar manner to that described for the grass plots.

2.2.2 Sample Processing and Microbial Parameters Measured

Soil samples were processed in the following manner:

1. samples were placed on a 2 mm sieve and as many roots as possible removed from the soil. These roots were washed, air dried and weighed, but not returned to the soil sample for the same reasons stated earlier.

2. dead grass litter was separated from the 0-5 cm soil and categorized as leaves or stems. These were air dried, weighed, chopped into 1-2 cm segments and returned to the soil sample since this litter would eventually form part of the soil system. Green plant material was removed from the sample and discarded.

3. as much soil as possible was sieved through the 2 mm mesh; stones and woody debris were discarded. Sewage sludge aggregates present in the sludge amended samples were pressed through the sieve so they would be included in the sample.

After sieving the samples, the effects of amendation on various soil and microbiological characteristics were investigated. The parameters which were studied in each of the three spoil types are listed in Tables 2 to 4. The techniques which were used for the various measurements are as follows:

1. numbers of bacteria - as described for the field study
2. numbers of actinomycetes - as described for the field study
3. hyphal lengths - as described for the field study.
4. loss on ignition - as described in Visser et al. (1984).
5. litter input and root wts - these were estimated from the air dried litter (stems and leaves) and roots sieved out of each sample. Since the area of each core or soil block was known, litter and root wts could be converted to g m^{-2}.
6. CO_2 efflux was measured in the same manner as that described for the field study. Samples were remoistened to the

Table 2. Soil and microbial characteristics which were investigated to determine the effects of amendation on a grassland coal mine spoil (tank study).

Parameter	Sampling times (mo after amendation)	Vegetation on sampled subplot	Sampling depth (cm)	
			0-5	5-15
Bacterial numbers	0.5, 15	Unvegetated	+	+
		Fall rye	+	+
Actinomycete numbers	0.5, 15	Unvegetated	+	+
		Fall rye	+	+
Loss on ignition	0.5, 15, 27	Unvegetated, Fall rye	+	+
Litter input, root wts	27	Fall rye	+	+ (roots only)
CO_2 evolution	0.5, 15, 27	Unvegetated, Fall rye	+	+
Microbial biomass C	0.5, 15, 27	Unvegetated, Fall rye	+	+
Filter paper decomposition	12, 24	Fall rye, alfalfa	+	
Wood dowel decomposition	12, 24	Fall rye, alfalfa	+	
Asymbiotic N_2 (C_2H_2) fixation	0.5	Preplanting (unvegetated)	+	+
Symbiotic N_2 (C_2H_2) fixation	3, 14, 26	Alfalfa	+	

Table 3. Soil and microbial characteristics which were investigated to determine the effects of amendation on a subalpine coal spoil (tank study).

Parameter	Sampling times (mo after amendation)	Vegetation on sampled subplot(s)	Sampling depth (cm)	
			0-5	5-15
Bacterial numbers	0.5, 15	Unvegetated, grass	+ +	+ +
Actinomycete numbers	0.5, 15	Unvegetated, grass	+ +	+ +
Hyphal lengths	0.5, 15	Unvegetated, grass	+	+
Loss on ignition	0.5, 27	Unvegetated, spruce	+	+
	0.5, 15, 27	Unvegetated, grass	+	+
Litter input, root wts	27, 39	Grass, spruce (roots only)	+	+ (roots)
CO_2 efflux	0.5, 15, 27	Unvegetated, grass	+	+
		Spruce	+	+
Microbial biomass C	0.5, 15, 27	Unvegetated, grass	+	+
	0.5, 27	Spruce	+	+
Filter paper decomposition	12, 24	Grass, spruce	+	
Wood dowel decomposition	12, 14	Grass, spruce	+	
Stem and leaf decomposition	(6)[1], 12	Grass, clover	+	
Asymbiotic N_2 (C_2H_2) fixation	0.5	Preplanting (unvegetated)	+	+

(cont'd)

Symbiotic N_2 (C_2H_2) fixation	3, 14, 26	Clover	+

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[1] Grass leaves sampled at 6 and 12 mo.

Table 4. Soil and microbial characteristics which were analyzed to determine the effects of amendation on a oil sand tailings (tank study).

Parameter	Sampling times (mo after amendation)	Vegetation on sampled subplot(s)	Sampling depth (cm)	
			0-5	5-15
Bacterial numbers	0.5, 15	Unvegetated, grass	+ +	+ +
Actinomycete numbers	0.5, 15	Unvegetated, grass	+ +	+ +
Hyphal length	0.5, 15	Unvegetated, grass	+	+
Loss on ignition	0.5, 27, 39	Unvegetated	+	+
	0.5, 15, 27, 39	Grass, jack pine	+	+
Litter input, root wt	27, 39	Grass, pine	+	+ (roots)
CO_2 efflux	0.5, 15, 27, 39	Unvegetated, grass pine	+	+
Microbial biomass C	0.5, 15, 27, 39	Unvegetated, grass pine	+	+
Filter paper decomposition	12, 24	Grass, sainfoin	+	
Wood dowel decomposition	12, 14	Grass, sainfoin	+	
Leaf and stem decomposition	(6)[1], 12	Grass, sainfoin	+	
Asymbiotic N_2 (C_2H_2) fixation	0.5	Preplanting (unvegetated)	+	+

(cont'd)

Symbiotic N_2 (C_2H_2) fixation	3, 14, 26	Sainfoin	+

---

[1] Grass leaves sampled at 6 and 12 mo.

moisture levels measured in the samples taken prior to planting (i.e. the first sample time at 0.5 mo) so that respiration measurements would be comparable amongst the various sampling times. The respiratory activity of the individual amendments was measured using a Gilson respirometer, since the liquid nature of the sewage sludge prevented this material from being processed on the CO_2 analyzer. Samples equivalent to 15 g dwt for the gypsum and 3 g dwt for the peat and sewage sludge were placed in Parkinson and Coups (1963) flasks and connected to the respirometer. Three, hourly measurements of O_2 uptake were made after samples had equilibrated for 24 h at 22°C.

7. microbial biomass was measured as described previously using the samples for which CO_2 efflux had been measured.

2.2.3 Decomposition Studies

2.2.3.1 Standard substrates (filter paper, wood dowel). The effects of amendation and vegetation types on the decomposition of cellulosic and lignified substrates were studied by following the decay of filter paper and fir wood dowel over a two year term (see Tables 2 to 4 for the vegetation types investigated). Filter papers and dowel segments were prepared in the same manner as for the field studies. One filter paper and one wood segment were placed around each of five stakes inserted in the appropriate subplots four months after planting. Two replicates of each substrate type were sampled after 12 mo incubation in the field while three replicates were sampled 24 mo after initiation of the experiment. Samples were processed as described previously and results expressed as % dry wt remaining.

In an effort to assess whether the decomposition potential of the particular vegetation types under investigation had altered since the initiation of the tank study, another set of filter papers were placed in the appropriate plots 28 mo after planting. Filters were prepared as described previously, but only two filters were placed in each plot. The % dry wt remaining was determined after 12 mo incubation in the field. Wood dowel was not used in this study since its weight loss was negligible after 24 mo in the field.

2.2.3.2 Plant residues (grass and legume litter). Litterbags (1 mm mesh and 16 cm x 8 cm) each containing either slender wheatgrass, alsike clover or sainfoin leaves or stems were placed in the subalpine and oil sand grass and legume plots, 28 mo after planting. The litter material used for this study was that clipped for standing crop estimates at the conclusion of the second growing season. Only plant material grown in the peat-treated plots was used in order to decrease variability in nutrient quality resulting from the different amendations. Plant material was dried to a constant weight at 35°C and weighed samples of stems or leaves were then placed in each bag. Leaves and stems were separated since stems tend to be more recalcitrant than leaves, therefore decomposing at a different rate than leaves do. Four bags each containing slender wheatgrass leaves, and two bags each containing grass stems were staked out in each of the grass subplots in the subalpine and oil sand tank plots. Two bags containing leaves (clover or sainfoin) and two bags containing stems (clover or sainfoin) were also placed in each of the alsike clover and sainfoin subplots. Slender wheatgrass leaves were sampled after 6 mo and 12 mo incubation in the field while all other substrate types were sampled 12 mo after placement in the field. Prior to removing the litter from the bags, samples were washed for 30 sec (15 sec on each side of bag) under cold running water in an effort to remove sand and debris adhering to the leaf and stem surfaces. Samples were then dried and % dry wt remaining was determined.

2.2.4 N_2 Fixation Studies

2.2.4.1 Asymbiotic N_2 (C_2H_2) fixation. Measurements of N_2 (C_2H_2) fixation of the variously amended spoils were conducted on samples removed immediately prior to planting the subplots. The acetylene reduction technique, as described for the field study, was used to determine N_2 fixation. Acetylene reduction was measured on sieved samples held at field moisture levels and on soil remoistened to field capacity. It was observed that N_2 (C_2H_2) fixation was extremely low and highly variable; hence measurements of this parameter were not continued.

2.2.4.2 Symbiotic N_2 (C_2H_2) fixation. The N_2 fixation capacity of rambler alfalfa (grassland spoil), alsike clover (subalpine spoil) and sainfoin (oil sands spoil) was determined at 3, 14 and 26 mo after planting. The plants were randomly chosen and, where possible, were either flowering or filling pods, since Hardy et al. (1971) observed with soybeans and peanuts that N_2 fixation increases with fruit formation and maturation. Plants, with as much root material as possible, were collected by digging a 20 x 20 x 40 cm deep soil block around each plant, and removing the whole block in an attempt to minimize disturbance of the roots. At least three plants were removed from each plot and transported to the laboratory where roots were washed out as quickly as possible on a 1 mm mesh screen. Excess water was blotted from the roots, shoots were separated from the roots and combined roots from each subplot placed in glass sealing jars. N_2 (C_2H_2) fixation was then determined using the same techniques as described previously. Empty jars injected with acetylene and jars containing roots with no acetylene served as controls to test for residual ethylene in the acetylene gas or natural ethylene production by the roots. After measuring the amount of acetylene reduced to ethylene by the root nodules, roots were dried at 80°C and N_2 (C_2H_2) fixation calculated as nmoles N_2 fixed g^{-1} dry root hr^{-1} using a C_2H_2/N_2 conversion factor of 3. Partial correlation coefficients were computed for data collected from alsike clover and sainfoin sampled 14 mo after planting to determine if any relationships existed between the N_2 (C_2H_2) fixing capacity of the plants and root wt, shoot wt, shoot N, total soil N and soil NO_3-N.

2.2.4 Statistical Analysis

Again (as mentioned for data collected in the field studies) many statistical techniques were required to analyze the data collected in the tank study. Therefore, to avoid confusion, the statistical tests required to analyze each data set have been footnoted on the tabulated data.

3. RESULTS

3.1. FIELD STUDY

3.1.1 Numbers of Bacteria and Actinomycetes and Hyphal Lengths

Data on the effects of surface mining on bacterial and actinomycete numbers and hyphal lengths at the three study sites are presented in Tables 5 to 7 and Fig. 1 and 2 (grassland and subalpine sites only). These results demonstrate that:

i) in the undisturbed soil at all three sites, numbers of bacteria were significantly higher in the surface organic layers of soil (i.e. soil from the A_h, H and FH horizons in the grassland, subalpine and oil sands plots respectively) than in the subsurface mineral horizons (i.e. B horizon soil at all three sites).

ii) mining disturbance resulted in an increase in bacterial numbers (particularly in the surface soil) at the grassland site. However, at the subalpine and oil sands sites, mining significantly reduced the numbers of bacteria in comparison with bacterial counts from the undisturbed surface soil horizons, but not in comparison with those from the subsurface mineral horizons.

iii) actinomycete numbers were significantly lower in the surface 0-5 cm deep soil from the disturbed plot than in the 0-15 cm undisturbed topsoil at the grassland site. Actinomycete counts in the 5-15 cm mineral soil were not affected by disturbance at this site.

iv) mining disturbance did not significantly influence actinomycete numbers at the subalpine site, while no actinomycetes were detected in soil from either the undisturbed or disturbed oil sands plots.

v) hyphal lengths, both total and those with cell contents, were significantly lower in the disturbed than undisturbed soil. At the subalpine site, mining also decreased the amount of mycelium with clamps (i.e. hyphae belonging to the basidiomycete group of fungi - a group which includes many of the ectomycorrhizal fungi). No clamped hyphae were observed in soil from the grassland

Table 5. Effect of surface mining on bacterial and actinomycete numbers and hyphal lengths at the grassland site.

Microbial Parameter	Undisturbed Topsoil (0-15)	Undisturbed Mineral (15-25)	Disturbed Mineral (0-5)	Disturbed Mineral (5-15)	MSE
Bacteria (x 10^6 g^{-1} dwt soil)	8.1^{ab2}	2.6^{a}	96.4^{c}	26.2^{bc}	0.79
Actinomycetes (x 10^5 g^{-1} dwt soil)	103.1^{b^1}	54.8^{ab}	6.0^{a}	114.1^{ab}	
Hyphal lengths (m g^{-1} dwt soil)					
Total	1201^{b}	1250^{b}	263^{a}	216^{a}	10484
With cell contents	127^{b2}	166^{b}	22^{a}	18^{a}	0.23

Data was analyzed by a one-way ANOVA. Where the F-test was significant, differences were determined using the Scheffé multiple contrast procedure (P = 0.05) (Neter and Wasserman, 1974). Values in each row followed by same letter(s) do not differ significantly. MSE = mean square error.

[1] Analyzed by a Kruskal-Wallis test.

[2] Data was ln Y transformed prior to analysis. Values presented are geometric means.

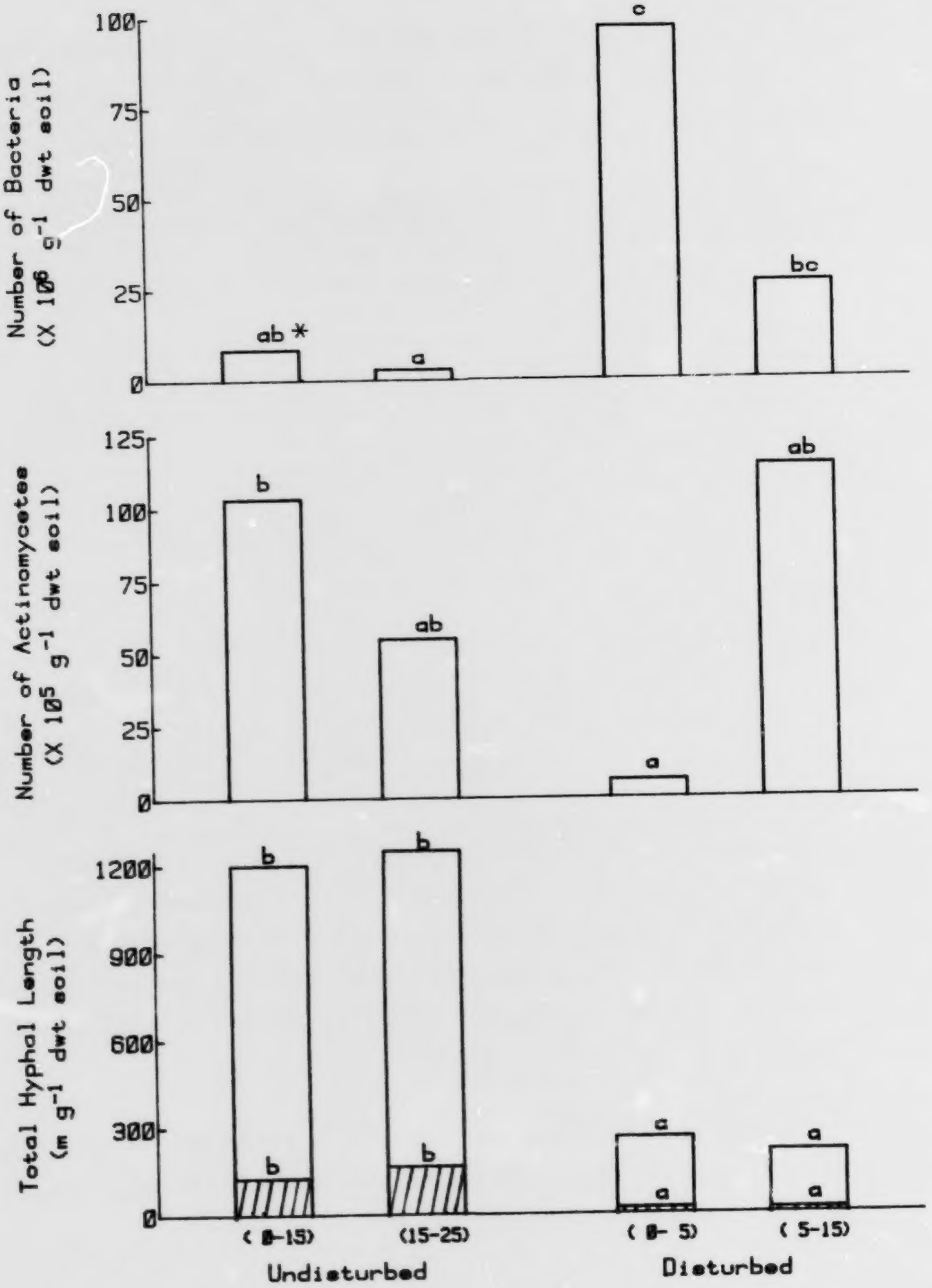

Fig. 1 Effect of surface mining on bacterial and actinomycete numbers and hyphal lengths at the grassland site.
▨ hyphal length with cell contents

*Bars headed by the same letter not statistically different

Table 6. Effect of surface mining on bacterial and actinomycete numbers and hyphal lengths in the subalpine soil[1].

	Soil and depth (cm)				
	Undisturbed		Disturbed		
Microbial Parameter	Organic (0-10)	Mineral (10-20)	Mineral (0-5)	Mineral (5-15)	MSE
Bacteria (x 10^6 g^{-1} dwt soil)	210^{c}	3.1^{a}	78.9^{b}	54.7^{b}	.21
Actinomycetes (x 10^5 g^{-1} dwt soil)	5.1^{a}	0^{a}[2]	1.47^{a}	1.84^{a}	2.77
Hyphal lengths (m g^{-1} dwt soil)					
Total	27106^{c}	2672^{b}	77^{a}	78^{a}	.10
With cell contents	4978^{c}	271^{b}	9^{a}	6^{a}	.41
With clamps	6488	106	.4	0	

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[1] Data was analyzed by a one-way ANOVA and Scheffé multiple contrasts. Bacteria and actinomycete data required a ln (y + 1) transformation while the hyphal length data required a ln Y transformation. Means for these parameters are geometric. Values in each row followed by the same letter do not differ significantly (p = 0.05). MSE = mean square error.

[2] No variation, therefore not included in analysis. The confidence interval calculated for the other treatments indicated this value was not significantly different from the other values.

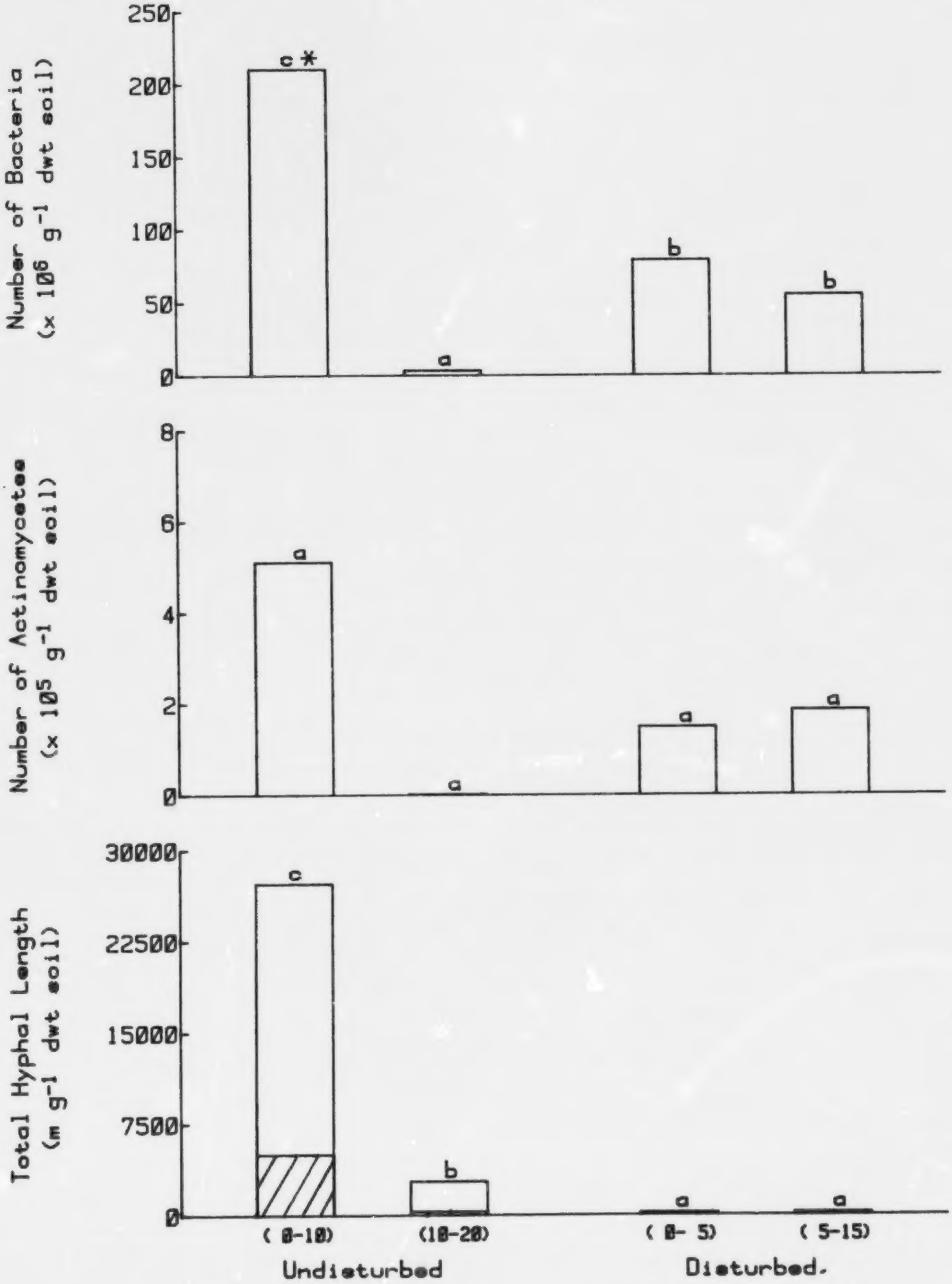

Fig. 2 Effect of surface mining on bacterial and actinomycete numbers and hyphal lengths in the subalpine soil.

▨ hyphal length with cell contents

*Bars headed by same letter are not statistically different

Table 7. Effect of surface mining on bacterial and actinomycete numbers in the extracted oil sands.

Microbial Parameter	Soil and depth (cm)			
	Undisturbed		Disturbed	
	Organic (0-5)	Mineral (5-15)	Mineral (0-5)	Mineral (5-15)
Bacteria[1] ($\times 10^6$ g^{-1} dwt soil)	34.3^b (28.8)	2.5^a (0.6)	7.5^a (0.7)	8.7^a (1.2)
Actinomycetes	0	0	0	0

[1] Data analyzed by Kruskal-Wallis test. Significant differences amongst the treatments were determined by post hoc comparisons ($p \leq 0.05$). (   ) = standard deviation.

site. Hyphal lengths in soil from the undisturbed oil sand plots were not determined.

3.1.2 Bacteria Isolated by Dilution Plating

The most frequently isolated groups of bacteria in the undisturbed prairie grasslands topsoil were *Arthrobacter*, *Bacillus*, the coryneforms and the Gram negative nonpigmented rods while *Bacillus*, the coryneforms and Gram negative nonpigmented rods were the most common organisms in the subsurface mineral soil (Table 8). After mining the grasslands soil, the isolation frequency of the coryneforms increased significantly (particularly when comparing the undisturbed topsoil with the disturbed soils) while the frequencies of *Bacillus* and the nonpigmented Gram negative rods decreased. In general, the frequency of Gram negative bacteria decreased and the Gram positive bacteria increased after mining. The % viability (after 6 mo storage) of the bacteria isolated from the topsoil was significantly lower than the % viability of isolates from the disturbed soils (Table 8).

The groups of bacteria isolated from the undisturbed subalpine soil were dominated by coryneforms, *Cytophaga*, *Flavobacterium* (organic soil only) and nonpigmented Gram negative bacteria (Table 9). As in the prairie grasslands soil, mining caused a significant increase in the frequency of coryneforms while the nonpigmented rods tended to decrease. The number of *Cytophaga* isolates was also lower in the disturbed than undisturbed soil. In general, the bacterial isolation data from the subalpine soils demonstrated the same trend as that from the grasslands soil; that is, Gram negative bacteria decreased while Gram positive bacteria increased after disturbance. The viability of isolates from the undisturbed subalpine soil was significantly affected by storage in comparison with the viability of isolates from the disturbed soils (Table 9).

The coryneforms and yellow pigmented and nonpigmented Gram negative rods were the most common bacteria in the undisturbed 0-5 cm soil from the oil sands site, while the coryneforms, *Cytophaga* and nonpigmented Gram negative rods were very frequent in the 5-15 cm

Table 8. Effect of surface mining on the isolation frequencies of the bacterial groups in grasslands minespoil[1].

	Percent of total viable isolates				
	Undisturbed		Disturbed		
Bacterial group	Topsoil (0-15)	Mineral (15-25)	Mineral (0-5)	Mineral (5-15)	MSE
Arthrobacter	13.9^{a}	3.3^{a}	5.0^{a}	10.0^{a}	81.5
Bacillus	29.6^{ab}	32.7^{b}	6.7^{ab}	3.3^{a}	246.0
Coryneforms (excluding *Arthrobacter* and *Nocardia*)	26.4^{a}	40.3^{ab}	71.7^{b}	68.3^{b}	472.4
Cytophaga	3.7	0	0	0	
	(5.05)	(0)	(0)	(0)	
Flavobacterium	2.0	0	0	0	
	(4.47)	(0)	(0)	(0)	
Nocardia	1.8	3.5	0	0	
	(4.07)	(4.77)	(0)	(0)	
Staphylococcus	0	0	6.7	0	
	(0)	(0)	(14.89)	(0)	
Yellow pigmented Gram negative rods	2	0	5.0	3.3	
	(4.47)	(0)	(7.46)	(4.55)	
Nonpigmented Gram negative rods	20.6^{b}	20.1^{b}	3.3^{ab}	0^{a}	74.6
Gram positive cocci	0	0	0	15	
	(0)	(0)	(0)	(12.35)	
Total Gram negative	28.2^{b}	20.3^{ab}	8.3^{a}	3.3^{a}	80.6
Total Gram positive	71.8^{a}	79.7^{ab}	91.6^{b}	96.7^{b}	80.6
% viability after storage	86.7^{a}	98.3^{ab}	100^{b}	100^{b}	43.4

(cont'd)

[1] Where possible, data was analyzed using a one-way ANOVA and Scheffé multiple contrasts. Values in each row followed by the same letter do not differ significantly ($p = 0.05$). MSE = mean square error. ( ) = standard deviation.

Table 9. Effect of surface mining on the isolation frequencies of the bacterial groups from the subalpine minespoil[1].

Bacterial group	Undisturbed		Disturbed		Stockpiled	
	Organic (0-10)	Mineral (10-20)	Mineral (0-5 cm)	Mineral (5-15 cm)	regolith	MSE
Arthrobacter[2]	5.4 (7.37)	3.3 (7.47)	17.6 (12.90)	18.5 (9.15)	0 (0)	
Bacillus	2.5^a	8.7^a	11.6^a	7.6^a	6.4^a	46.0
Chromobacterium	0 (0)	0 (0)	0 (0)	0 (0)	3.0 (5.25)	
Coryneforms (excluding *Arthrobacter*)	6.0^a	14.4^{ab}	46.1^c	27.6^{abc}	19.1^{abc}	272.2
Cytophaga	16.2 (10.26)	22.9 (20.34)	0 (0)	5.6 (8.25)	3.0 (5.77)	
Flavobacterium	11.1 (12.10)	0 (0)	1.7 (3.71)	7.2 (7.17)	0 (0)	
Flexibacter	0 (0)	0 (0)	0 (0)	1.8 (4.07)	0 (0)	
Nocardia	0 (0)	0 (0)	5.9 (5.41)	2.0 (4.47)	3.0 (5.25)	
Pseudomonas	2.0 (4.47)	0 (0)	0 (0)	0 (0)	0 (0)	

Staphylococcus	2.0	2.5	3.3	4.0	3.6	
	(4.47)	(8.35)	(7.47)	(8.94)	(10.57)	
Other Gram positive cocci	0	0	6.8	1.8	6.1	
	(0)	(0)	(7.04)	(4.07)	(17.32)	
Yellow pigmented	7.0	3.3	0	5.6	3.3	
Gram-negative rods	(10.95)	(7.47)	(0)	(8.25)	(5.77)	
Orange pigmented	0	0	0	0	2.3	
Gram-negative rods	(0)	(0)	(0)	(0)	(4.79)	
Nonpigmented Gram-negative rods[3]	47.9^{b}	44.9^{ab}	4.3^{a}	15.0^{a}	34.7^{ab}	0.3
Gram negative bacteria	84.2^{c}	71.1^{ab}	8.8^{a}	36.5^{ab}	55.4^{abc}	397.9
Gram positive bacteria	15.9^{a}	29.0^{ab}	91.3^{c}	61.5^{bc}	44.6^{abc}	383.7
% viability after storage	58.3^{a}	58.3^{a}	91.7^{b}	90.0^{b}	91.7^{b}	134.5

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[1] Where possible, data was analyzed by a one-way ANOVA and Scheffé multiple contrasts. Values in each row followed by same letter do not differ significantly ($p = 0.05$). MSE = mean square error. ( ) = standard deviation.

[2] No Arthrobacter occurred in regolith, hence this treatment was not included in the analysis. Although the F value was significant for the other treatments, no pairwise or linear trends could be detected.

[3] Data was 2 arcsin $\sqrt{p}$ transformed. Means are geometric.

deep soil from the same site (Table 10). The bacterial groups present in the sand after extraction of the oil were not determined in this study.

3.1.3 Fungi Isolated by Soil Washing Technique

Tables 11 to 13 present data on the most frequently isolated fungi from the three study sites, both before and after disturbance. At the grassland site, the Chrysosporium-Pseudogymnoascus spp., and the sterile dark and sterile hyaline forms (these are organisms which will not fruit in culture; hence are termed sterile) were the most common isolates from the undisturbed soil (Table 11). Fungal isolates from the disturbed soil were dominated by Alternaria spp., Cladosporium spp., Penicillium spp., sterile dark forms and yeasts. The % of total soil particles plated which yielded fungi ranged from 70 to 90.

Mortierella spp., Mucor spp., Mycelium radicis atrovirens (MRA), Oidiodendron spp. (organic soil mainly), Penicillium spp. (organic soil mainly), Tolypocladium (mineral soil), Trichoderma polysporum and the yeasts (mineral soil mainly) formed a large proportion of the isolates from the undisturbed spruce-fir forest at the subalpine site (see Table 12). The fungal community in the stockpiled regolith consisted mainly of Acremonium spp., Chrysosporium spp., Phialophora spp. and yeasts. After the stockpiled regolith had been spread, but prior to planting, the frequency of Candida (a yeast) increased significantly. There were also significantly more isolates of Chrysosporium spp. in the disturbed soil than in the undisturbed organic soil. Therefore, the effect of mining disturbance on the fungal community of the subalpine soils was to shift it from one dominated by Mortierella, Mucor, Oidiodendron, Penicillium and Trichoderma polysporum to one dominated by the Candida and Chrysosporium spp. The % of total soil particles plated from the disturbed plot which yielded fungi was significantly lower than that from the undisturbed soils.

Of the three study sites, the effects of mining on the fungal community were most drastic at the oil sands (Table 13). The

Table 10. Isolation frequencies of the bacterial groups from the undisturbed oil sands site[1].

Bacterial group	Percent of total viable isolates			
	Organic (0-5 cm)		Mineral (5-15 cm)	
	$\bar{X}$	SD	$\bar{X}$	SD
Arthrobacter	1.4	3.2	6.2	9.1
Bacillus	2.9	3.9	0	0
Chromobacterium	1.4	3.2	0	0
Coryneforms (excluding *Arthrobacter*)	30.8^a	11.8	24.8^a	19.6
Cytophaga	8.5	15.5	22.8	37.6
Flavobacterium	2.8	3.8	3.6	8.1
Yellow pigmented Gram negative rods	16.9	16.4	9.9	11.3
Orange pigmented Gram negative rods	4.2	3.8	0	0
Nonpigmented Gram negative rods	31.2^a	20.8	32.6^a	31.8
Gram negative bacteria	65.0^a	13.5	69.0^a	20.1
Gram positive bacteria	35.0^a	13.5	31.0^a	20.1
% viability after storage	94.7^b	3.0	68.0^a	24.2

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[1] Where possible data were analyzed by Student t-test. Values in each row followed by same letter do not differ significantly ($p = 0.05$). SD = standard deviation.

[2] Data analyzed by Fisher's randomization t-test.

Table 11. Effect of surface mining on the per cent frequency of occurrence of the most commonly isolated fungal groups at the prairie grasslands site (i.e. having a mean frequency of occurrence >5% in any one soil replicate).

	Soils and depth (cm)				
	Undisturbed		Disturbed		
Fungal group	Topsoil (0-15)	Mineral (15-25)	Mineral (0-5)	Mineral (5-15)	MSE
Alternaria spp.	0^{a}	0^{a}	23.7^{a}	9.7^{a}	53.51[3]
Chrysosporium-Pseudogymnoascus	20.0^{ab}[1]	66.8^{b}	3.3^{a}	7.3^{a}	0.26
Cladosporium spp.	0.3^{a}	0^{a}	13.7^{b}	2.1^{ab}	0.08
Fusarium spp.	3.8^{ab}	0.1^{a}	1.6^{ab}	5.3^{b}	0.04
Mortierella spp.	4.5^{a}[1]	6.3^{a}	0.1^{a}	1.8^{a}	0.06
Oidiodendron spp.	0^{a}	0^{a}	5.5^{a}	9.2^{a}	21.53[3]
Penicillium spp.	0.6^{a}	4.9^{ab}	6.4^{ab}	23.1^{b}	0.13
Trichocladium sp.	0^{a}	6.0^{a}	0^{a}	0^{a}	12.4[3]
Sterile dark	24.0^{a}	4.0^{a}	17.5^{a}	12.5^{a}	121.77
Sterile hyaline	7.5^{ab}	15.2^{b}	1.7^{a}	5.0^{a}	23.0
Yeasts	3.5^{ab}[2]	0.7^{a}	35.2^{b}	12.5^{ab}	
% of particles plated which yielded fungi	76.0	86.8	70.0	87.5	

Values in each row followed by same letter do not differ significantly ($p = 0.05$).

[1] Data analyzed by a one-way ANOVA after 2 arcsin $\sqrt{p}$ transformation (sterile hyaline data did not require a transformation). Significant differences were detected by Scheffé multiple contrasts. Values are geometric means. MSE = mean square error.

[2] Data analyzed with a Kruskal-Wallis test.

[3] Data analyzed by Student t-test; except for *Trichocladium*. Value in MSE column is 95% confidence interval for difference.

Table 12. Effect of surface mining on the per cent frequency of occurrence of the most commonly isolated fungi at the subalpine site (i.e. those genera having a mean frequency of occurrence >5% in any one soil replicate)[1].

Fungus	Soils and depth (cm)				Stockpiled regolith	MSE
	Undisturbed		Disturbed			
	Organic (0-10)	Mineral (10-20)	Mineral (0-5)	Mineral (5-15)		
Acremonium	5.3^{b}	1.8^{ab}	0.3^{a}	0.5^{ab}	11.1^{c}	.031
Candida[2]	0	0	20.3^{a}	9.3^{a}	0	
Chrysosporium	3.3^{a}	8.8^{ab}	18.0^{b}	21.5^{b}	21.1^{b}	.089
Mortierella	84.0^{c}	35.0^{b}	0.3^{a}	2.5^{a}	0	.080
Mucor	14.0^{ab}	27.3^{b}	0.5^{a}	1.5^{a}	1.1^{a}	.177
Mycelium radicis atrovirens	34.7	6.0	0	0	0	
Oidiodendron	20.0^{b}	4.0^{ab}	1.3^{a}	0.3^{a}	3.3^{ab}	.090
Penicillium	54.7^{b}	8.5^{a}	0.8^{a}	0.5^{a}	1.1^{a}	.308
Phialophora	0	9.0	0.5	0.3	30.0	
Pseudogymnoascus bhattii	0	5.0	0	0.5	0	
Rhinocladiella	11.3	3.8	2.0	0.3	0	
Tolypocladium	2.7	16.8	0	0	0	
Trichoderma polysporum	26.7	14.5	0	0	0	
Verticillium	7.3	0	0	0	0	

(cont'd)

Yeasts	0	19.3	2.0	11.5	43.3	
Sterile dark	2.2^{a}	9.5^{a}	5.5^{a}	3.0^{a}	8.9^{a}	.080
Sterile hyaline	0	8.3	1.3	0	6.7	
% total plated soil particles yielding fungi	100.0^{c}	98.5^{c}	45.4^{ab}	40.2^{a}	82.3^{bc}	378.1

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[1] Frequency of occurrence = $\frac{\text{number of isolates per genus}}{\text{total number of plated soil particles}}$ x 100%. Where possible, data was analyzed by a one-way ANOVA after 2 arcsin $\sqrt{p}$ transformation. Transformed data presented as geometric means. Significant differences were detected by Scheffé multiple contrasts for pairwise comparisons. Values in each row followed by the same letter do not differ significantly ($p \leq 0.05$). MSE = mean square error.

[2] Data analyzed by Student t-test.

Table 13. Effect of surface mining on the percent frequency of occurrence of the most commonly isolated fungal genera at the oil sands site (i.e. those having a mean frequency of occurrence >5% in any one soil replicate)^1.

Fungus	Soils and depth (cm)				MSE
	Undisturbed		Disturbed		
	Organic (0-5)	Mineral (5-15)	Mineral (0-5)	Mineral (5-15)	
Acremonium^2	10.0^a (14.5)	0	1.1 (1.9)^a	0	
Beauveria	0	10.0 (22.4)	0	0	
Cladosporium	0	0	10.0 (8.8)	0	
Humicola	0	15.3 (6.1)	0	0	
Mortierella	68.5^b	10.0^a	2.2^a	0	122.1
Mucor	13.0 (7.8)	10.7 (4.3)	0	0	
Mycelium radicis atrovirens	2.5 (4.3)	10.7 (10.4)	0	0	
Oidiodendron	32.5 (24.2)	7.3 (3.6)	0	0	
Penicillium	86.5^b	7.3^a	1.1^a	0	59.3
Pseudogymnoascus	0	16.0 (8.6)	0	0	
Thysanophora	2.5 (5.6)	15.3 (13.0)	0	0	
Trichoderma	44.2 (37.8)	14.7 (21.9)	0	0	
Verticillium	6.0 (5.5)	0	0	0	
Yeasts	0	2.0^a	10.0^a	1.1^a	41.4
Sterile dark	2.0^a	8.7^b	2.2^a	0	10.52

(cont'd)

Table 13. (cont'd)

Sterile hyaline[2]	0	12.7^{a} (7.2)	2.2^{a} (1.9)	0	
% total plated soil[3] particles yielding fungi	99.7^{c}	96.4^{c}	26.5^{b}	0.4^{a}	192.05

---

[1] Frequency of occurrence = $\frac{\text{number of isolates per genus}}{\text{total number of plated soil particles}} \times 100\%$. Where possible, data were analyzed by a one-way ANOVA and Scheffé multiple contrasts for pairwise comparisons. (0 values were excluded from the analysis). Values in each row followed by the same letter are not significantly different ($p \leq 0.05$). MSE = mean square error.

[1] Data analyzed by a Student t-test. ( ) = standard deviation.

[3] Data were 2 arcsin $\sqrt{p}$ transformed for analysis. Means are geometric.

fungi isolated from the undisturbed jack pine woodland were highly diverse with Mortierella, Oidiodendron, Penicillium and Trichoderma being the most common fungi in the organic soil and Humicola, Pseudogymnoascus, Thysanophora, Trichoderma and sterile hyaline forms, the most common in the subsurface mineral soil. The tailing sand, however, was very poorly colonized by fungi. Only the surface, 0-5 cm sand was colonized to any degree - the organisms being mainly Cladosporium spp. and yeasts which are both common in the atmosphere. Data on the % total soil particles plated yielding fungi provide further evidence for the sparsity of fungi in the sand after oil extraction.

3.1.4 Microbial Activity, Biomass and ATP

At the grassland and subalpine sites surface mining resulted in a significant decrease in microbial activity (CO_2 efflux), microbial biomass C and ATP levels (Tables 14 and 15, Figs. 3 and 4). Although microbial biomass C measurements were higher in the undisturbed sand than in the disturbed sand, mining did not significantly influence levels of microbial activity and ATP (Table 16, Fig. 5). Data for these two parameters, however, were exceedingly variable.

3.1.5 Asymbiotic N_2 Fixation

Measurements of asymbiotic N_2 fixation in remoistened soil from the grassland and subalpine site were extremely low in all soils tested and highly variable (Tables 17 and 18). No N_2 (C_2H_2) fixation was measured in the disturbed grassland soils and very low levels of activity were recorded for the disturbed soils and stockpiled regolith from the subalpine site. Asymbiotic N_2 fixation was not assessed for soils from the oil sands site.

3.1.6 Decay Rates of Standard Substrates (Filter Paper, Wood Dowel)

Values for the % dry wt remaining of filter paper placed in the undisturbed and disturbed grassland plots and sampled regularly

Table 14. Effect of surface mining on CO_2 efflux, microbial biomass C and ATP levels at the grassland site.

Microbial parameter	Soil type and depth (cm)			
	Undisturbed topsoil (0-15)		Disturbed mineral (0-5)	
	$\bar{X}$	SD	$\bar{X}$	SD
CO_2 evolution (ml $100g^{-1}$ dry soil hr^{-1})	0.43^{a}[1]	0.05	0.16^{b}	0.07
Microbial biomass C (mg $100g^{-1}$ dry soil)	84.6^{a}	9.6	14.8^{b}	8.3
ATP (μg $100g^{-1}$ dry soil)	32.2^{a}[2]	40.0	1.4^{b}	1.8
Soil moisture (% wet wt)	13.0^{a}	0.53	12.0^{a}	1.64

[1] $CO_2 \uparrow$ data and microbial biomass data were analyzed using a Student t-test. Means ($\bar{X}$) in each row followed by the same letter do not differ significantly ($p = 0.05$). SD = standard deviation.

[2] The Wilcoxon test was applied to the ATP data. Values in each row followed by the same letter do not differ significantly ($p = 0.05$).

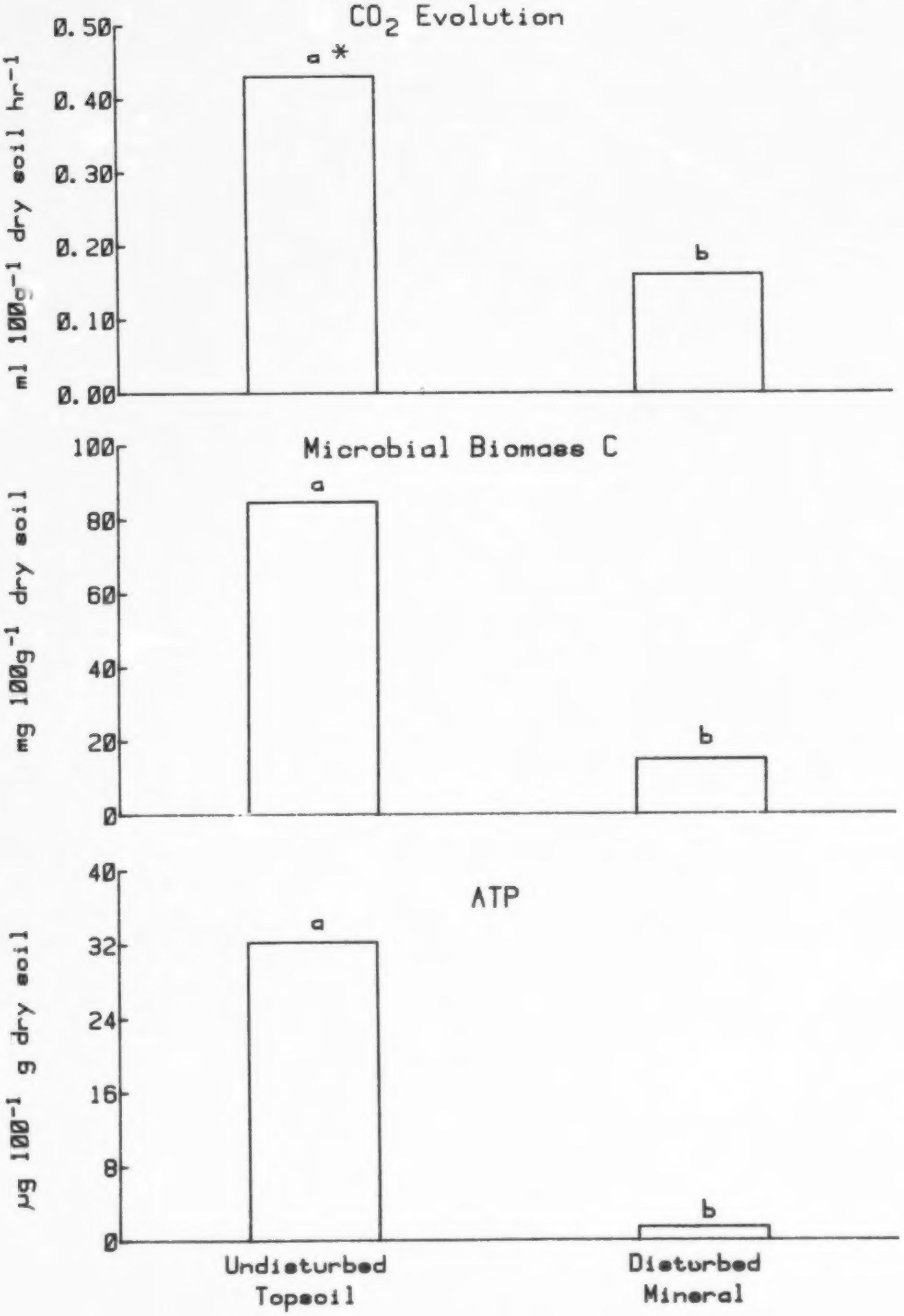

Fig. 3 Effect of surface mining on CO_2 efflux, microbial biomass C and ATP levels at the grassland site. *Bars headed by same letter are not statistically different

Table 15. Effect of surface mining on CO_2 efflux microbial biomass C and ATP levels at the subalpine site[1].

Soil type and depth (cm)	Microbial Parameter							
	CO_2 efflux (ml 100 g^{-1} soil hr^{-1})		Microbial biomass (mg C 100 g^{-1} soil)		ATP (μg 100 g^{-1} soil)		Soil H_2O (% wet wt)	
	$\bar{X}$	SD	$\bar{X}$	SD	$\bar{X}$	SD	$\bar{X}$	SD
Undisturbed organic (0-10)	5.29^b	.07	978.44^b	.77	20.32^b	64.05	61	9.00
Disturbed mineral (0-5)	0.14^a	.07	24.01^a	1.07	1.27^a	.02	7	1.47
Undisturbed mineral (10-20)	0.55^b	0	66.05^b	1.76	2.94^a	.75	41	.43
Disturbed mineral (5-15)	0.14^a	.32[2]	32.80^a	4.11	$.24^a$	.04	10	.79

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[1] Data analyzed using a Student t-test. Undisturbed organic biomass required sq. rt. transformation and ATP data was ln Y transformed. Transformed data are presented as geometric means. Means in each column within a soil layer followed by the same letter do not differ significantly ($p = 0.05$). SD = standard deviation.

[2] Undisturbed mineral data had 0 variance, hence means were tested using the 95% confidence interval for the mineral data.

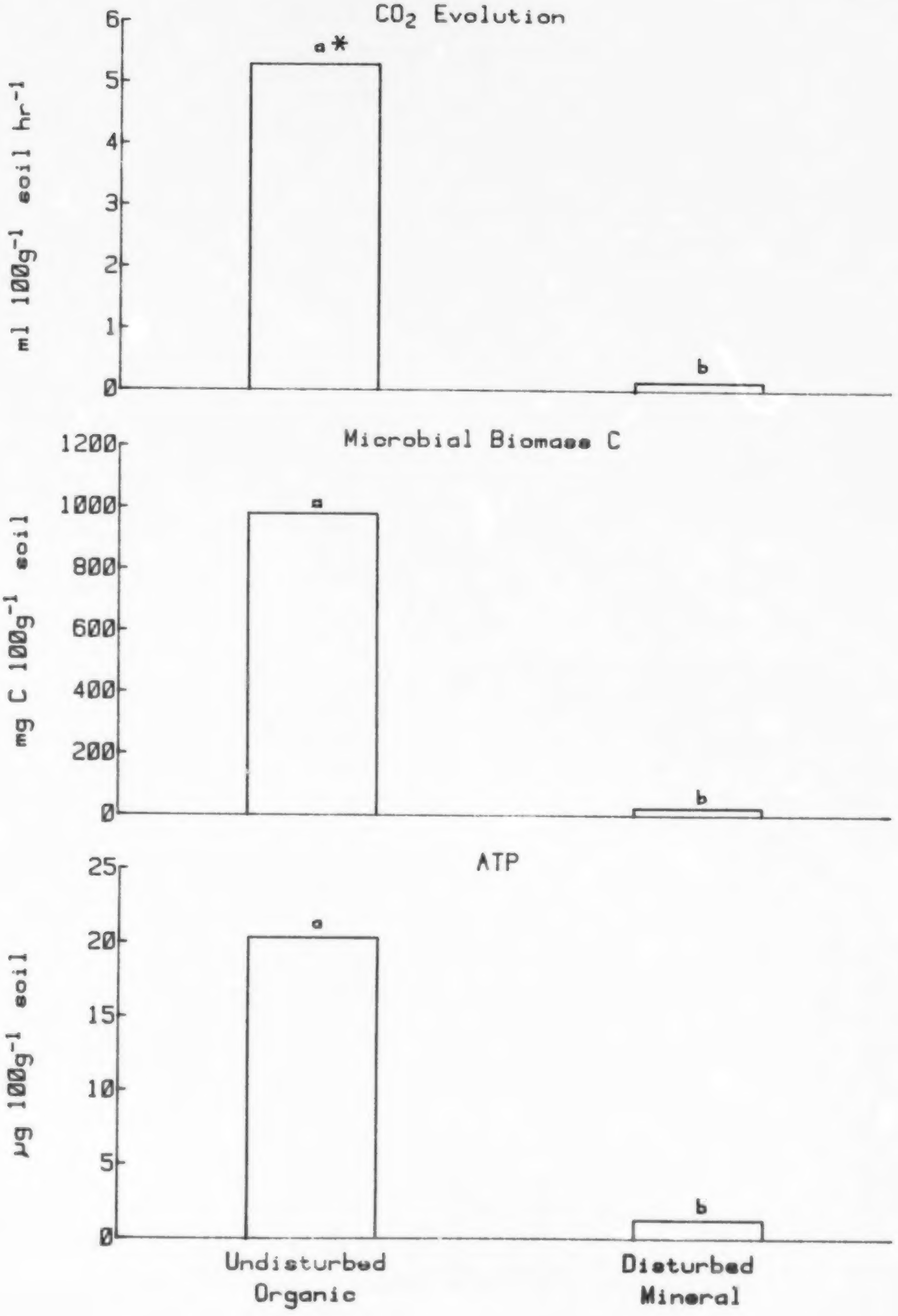

Fig. 4 Effect of surface mining on CO_2 efflux, microbial biomass and ATP levels at the subalpine site.
*Bars headed by same letter are not statistically different

Table 16. Effect of surface mining on CO_2 efflux, microbial biomass C and ATP levels at the oil sands site[1].

Soil type and depth (cm)	Microbial Parameter							
	CO_2 efflux (ml 100 g^{-1} soil hr^{-1})		Microbial biomass (mg C 100 g^{-1} soil)		ATP (μg 100 g^{-1} soil)		Soil H_2O (% wet wt)	
	$\bar{X}$	SD	$\bar{X}$	SD	$\bar{X}$	SD	$\bar{X}$	SD
Undisturbed organic (0-5)	.10^{a}[2]	.32	21.44^{b}	.36	3.16	3.98	6.3	2.7
Disturbed mineral (0-5)	.05^{a}	0	3.61^{a}	.28	0	0	5.2	1.4
Undisturbed mineral (5-15)	.10^{a}	.12	9.28^{b}	1.52	0	0	5.3	1.3
Disturbed mineral (5-15)	.05^{a}	0	3.97^{a}	.64	0	0	6.7	2.1

---

[1] Data analyzed by a Student t-test. Disturbed basal CO_2 ↑ data had no variance so the mean was used as an absolute value and tested with 95% confidence interval. Values in each column within the same soil layer followed by the same letter do not differ significantly ($p = 0.05$).

[2] Required a sq. rt. transformation. Means are geometric.

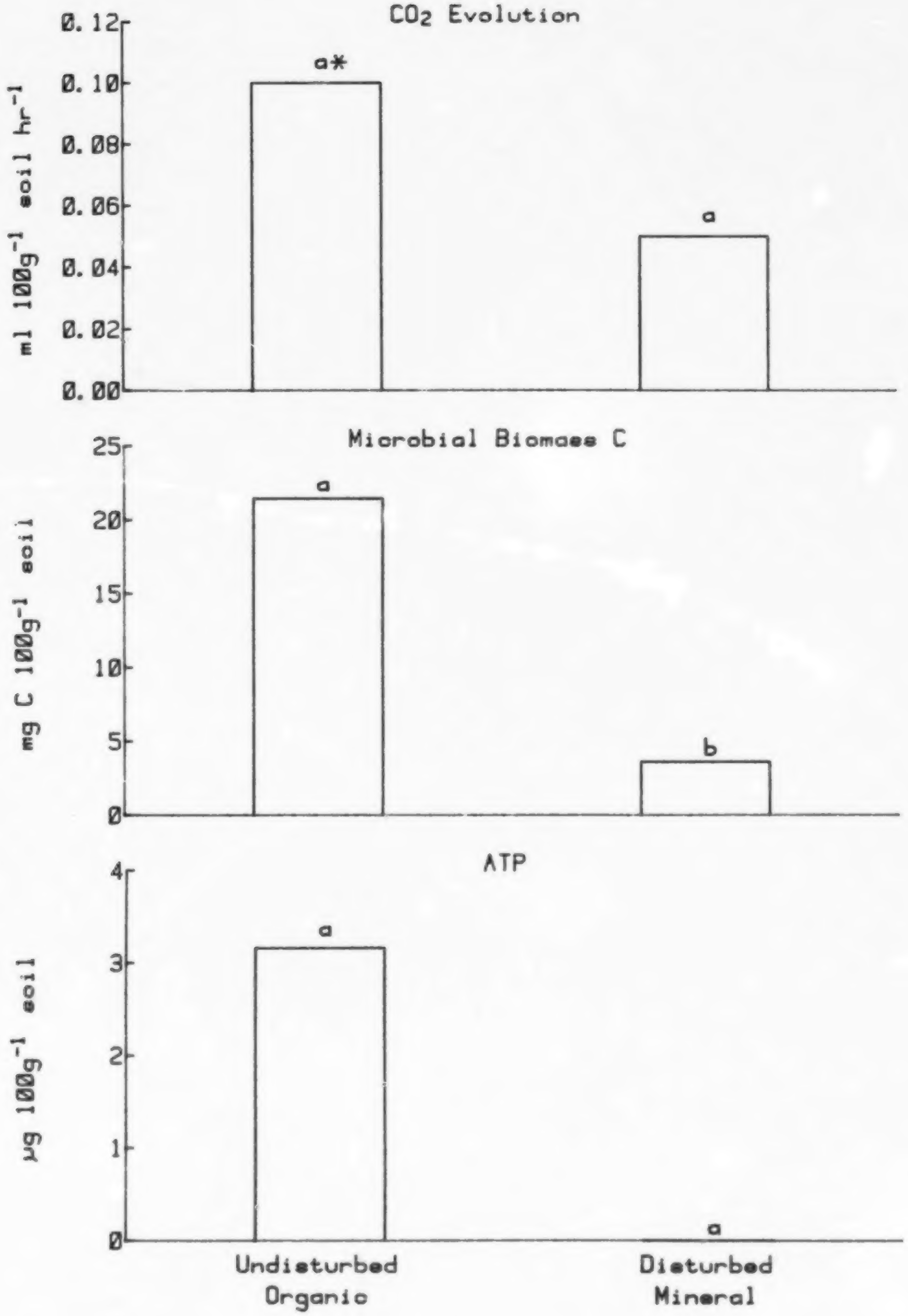

Fig. 5 Effect of surface mining on CO_2 efflux, microbial biomass and ATP levels at the oil sands site.
*Bars headed by same letter are not statistically different

Table 17. Effect of surface mining on N_2 (C_2H_2) fixing potential of remoistened soil from the grassland site.

Parameter		Soil and depth (cm)			
		Undisturbed		Disturbed	
		Topsoil (0-15)	Mineral (15-25)	Mineral (0-5)	Mineral (5-15)
Moisture (% wet wt)	$\bar{x}$	33	27	32	32
	SD	1	1	1	1
N_2 (C_2H_2) fixation[1]	$\bar{x}$	.15	.21	0	0
(nmol.100g^{-1} dry soil hr^{-1})	SD	.16	.11	0	0

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[1] The C_2H_2/N_2 conversion factor was assumed to be 3 (Hardy et al., 1973).

Table 18. Effect of surface mining on N_2 (C_2H_2) fixing potential of remoistened soil from the subalpine site.

Parameter		Soil and depth (cm)				
		Undisturbed		Disturbed		Stockpiled regolith
		Organic (0-10)	Mineral (10-20)	Mineral (0-5)	Mineral (5-15)	
Moisture (% wet wt)	$\bar{X}$	78.6	39.5	19.1	14.4	19.3
	SD	1.4	9.8	2.6	0.9	0.8
N_2 (C_2H_2) fixation[1] (nmol.100 g^{-1} dwt soil hr^{-1})	$\bar{X}$	.30	.01	.11	.05	.02
	SD	.28	.03	.13	.05	.03

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[1] The C_2H_2/N_2 conversion factor was assumed to be 3 (Hardy et al., 1973). SD = standard deviation.

thereafter are presented in Table 19 and Fig. 6. There were no differences in wt loss of filters between the undisturbed and disturbed plots until 24 mo after the initiation of the experiment when the disturbed plot filters had lost more wt than the undisturbed plot filters. However, this difference was not significant until 44 mo after the filters were placed on the plots. At that time the wt of paper remaining on the disturbed soil was negligible while 72% of the paper remained in the undisturbed plot.

The decomposition of filter paper placed in the undisturbed and disturbed plots at the subalpine site followed a similar trend to that observed in the grassland site (Table 20, Fig. 7). Filters decayed more rapidly at the disturbed than undisturbed site, but this difference did not become obvious until 33 mo after the initiation of the experiment.

Since no long term decomposition experiment was established on a disturbed plot at the oil sands, filter paper wt loss after 1 yr incubation in the undisturbed jack pine plot was compared with the 1 yr wt loss obtained for filters placed in the control plots in the tank study. The data obtained (Table 21) demonstrated that wt loss of filter paper over the short term was not significantly different between undisturbed and disturbed plots.

Amendation of filter paper with ammonium nitrate to decrease the C/N ratio, resulted in significantly faster decay of the filters placed at the undisturbed grassland site, but had no significant effects on decay rates of filters placed in the disturbed grassland plot and both disturbed and undisturbed subalpine plots (Tables 22, 23, 24 and 25).

The wt loss of fir dowel at the grassland and subalpine sites was negligible over the 44, 45 mo term of the study (Tables 26 and 27). No differences in dowel decomposition were observed between disturbed and undisturbed plots at any of the study sites (Tables 21, 26 and 27) and amendation of the wood with ammonium nitrate did not appear to hasten the rate of decay (Tables 26 and 27). Blocks of spruce wood placed at the subalpine site also decomposed very slowly

Table 19. Effect of surface mining on the decomposition (% dry wt remaining) of cellulose filter paper at the prairie grassland site[1].

Time (mo)	Site	
	Undisturbed	Disturbed
6	99^{d}	100^{d}
12	99^{d}	97^{d}
20	99^{d}	99^{d}
24	93^{cd}	51^{bc}
32	87^{bcd}	26^{ab}
44	72^{bcd}	0.4^{a}

[1] Data analyzed by a two-way ANOVA after 2 arcsin $\sqrt{p}$ transformation. MSE = .1710. A significant interaction was observed ($p \leq .001$). Scheffé multiple contrasts for pairwise comparisons were then calculated to detect significant differences in treatment means. Values are geometric means and where followed by the same letter(s) do not differ significantly ($p \leq 0.05$).

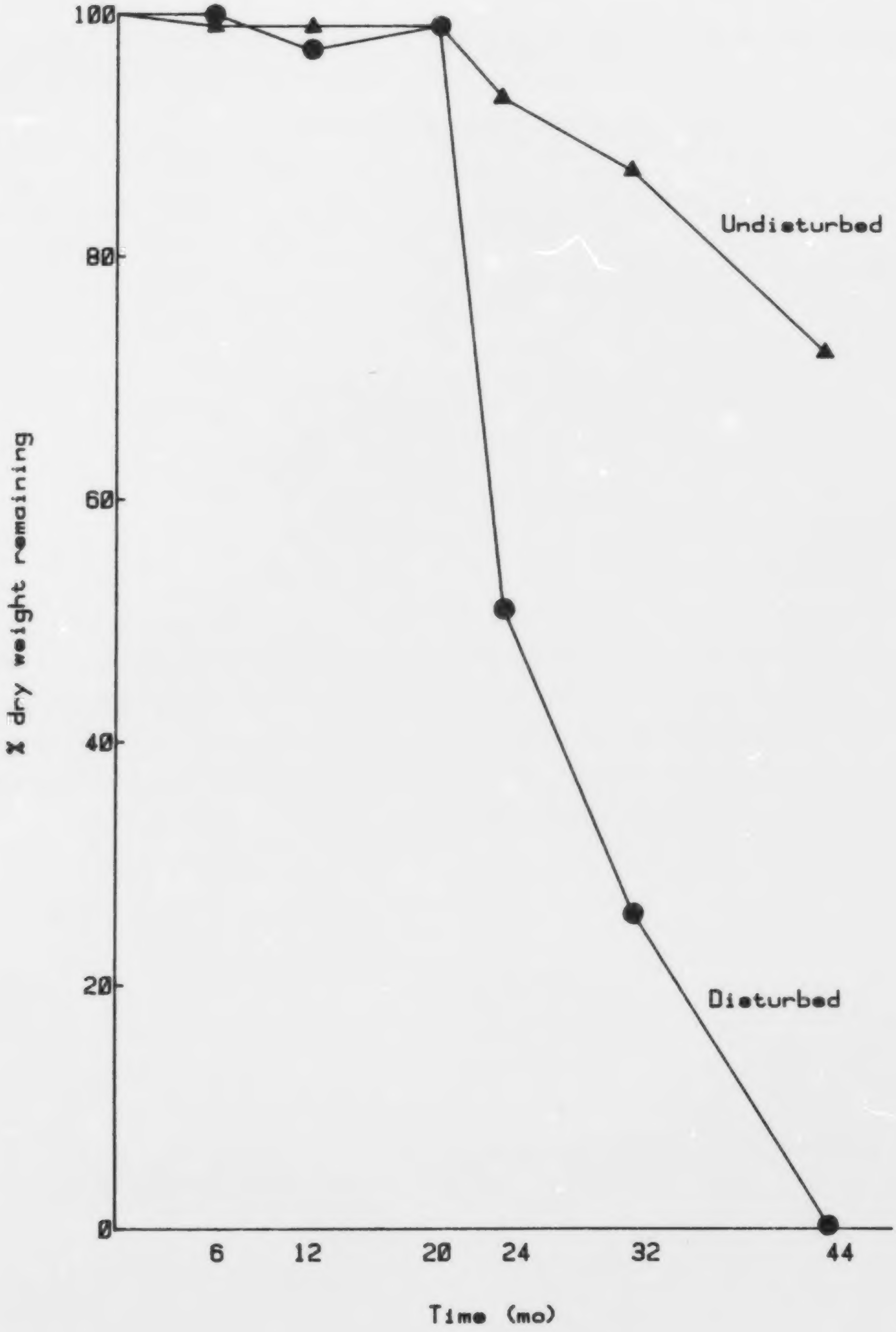

Fig. 6 Effect of surface mining on the decomposition of cellulose filter paper at the grassland site.

Table 20. Effect of surface mining disturbance on the decomposition of unamended filter paper at the subalpine site[1].

Time (mo)	% dry filter remaining		Time $\bar{X}$
	Undisturbed site	Disturbed site	
9	100	92	97^b
12	99	93	97^b
21	100	92	98^b
24	84	84	84^{ab}
33	86	53	69^{ab}
36	63	51	57^a
45	61	19	39^a
Site $\bar{X}$	90^b	72^a	

---

[1] Data analyzed by a two-way ANOVA (MSE = 1501.18) and Scheffé multiple contrasts for pairwise comparisons. Values in the time $\bar{X}$ column or site $\bar{X}$ row followed by the same letter(s) do not differ significantly ($p \leq 0.05$).

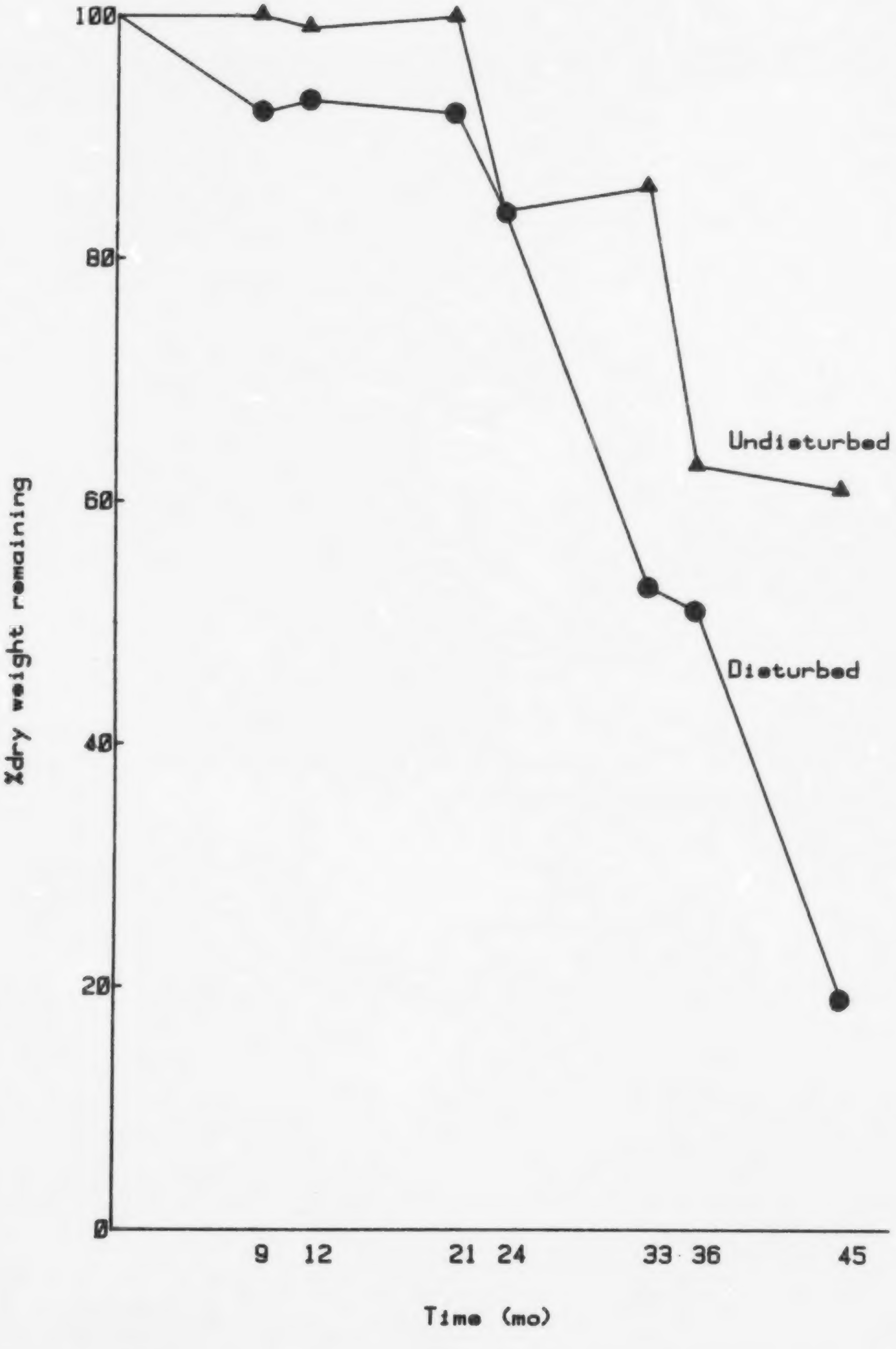

Fig. 7 Effect of surface mining on the decomposition of cellulose filter paper at the subalpine site.

Table 21. Effect of mining disturbance on the decomposition of filter paper and fir dowel after 1 yr on the oil sands site[1].

Substrate	Site	
	Undisturbed	Disturbed (tanks)
Filter paper	87^a	85^a
Fir dowel[2]	96	99

[1] Data analyzed by Student t-test. Values followed by same letter do not differ significantly ($p \leq 0.05$).

[2] Data not analyzed due to lack of variation.

Table 22. Decomposition (% weight remaining) of ammonium nitrate amended and unamended filter papers at the undisturbed prairie grassland plots[1].

	Treatment		
Time (mo)	Unamended	N-amended	Time $\bar{X}$
6	99	92	96^{dc}
12	99	95	98^{d}
20	99	90	96^{dc}
24	93	83	88^{bc}
32	87	82	85^{b}
44	72	63	67^{a}
Treatment $\bar{X}$	94^{b}	85^{a}	

---

[1] Data analyzed by a two-way ANOVA after 2 arcsin $\sqrt{p}$ transformation (MSE = .0638) and Scheffé multiple contrasts for pairwise comparisons. Values in the time $\bar{X}$ column or treatment $\bar{X}$ row followed by the same letter(s) do not differ significantly ($p \leq 0.05$). Values are geometric means.

Table 23. Decomposition (% dry wt remaining) of ammonium nitrate amended and unamended filter papers at the disturbed prairie grassland plots[1].

Time (mo)	Treatment Unamended	 Amended	Time $\bar{X}$
6	100	93	98^{d}
12	97	86	93^{d}
20	99	80	92^{d}
24	51	68	60^{c}
32	26	36	31^{bc}
44	0.4	2.0	1.0^{a}
Treatment $\bar{X}$	68^{a}	60^{a}	

---

[1] Data analyzed by a two-way ANOVA after a 2 arcsin $\sqrt{p}$ transformation (MSE = .2830) and Scheffé multiple contrasts for pairwise comparisons. Values in the time $\bar{X}$ column or treatment $\bar{X}$ row followed by the same letter(s) do not differ significantly ($p \leq 0.05$). Values are geometric means.

Table 24. Decomposition of unamended and N-amended filter papers at the undisturbed subalpine site[1].

Time (mo)	% dry wt. remaining	
	Unamended	N-amended
9	100^{d}	91^{abcd}
12	99^{bcd}	83^{abcd}
21	100^{d}	88^{abcd}
24	84^{abcd}	72^{abc}
33	86^{abcd}	84^{abcd}
36	63^{a}	67^{ab}
45	61^{a}	68^{ab}

---

[1] Data analyzed by two-way ANOVA (MSE = 396.61). A significant interaction was observed so Scheffé multiple contrasts were applied to all values to determine treatment effects. Values followed by the same letter(s) do not differ significantly ($p \leq 0.05$).

Table 25. Decomposition of unamended and N-amended filter papers at the disturbed subalpine sites[1].

Time (mo)	% dry filter paper remaining		Time $\bar{X}$
	Unamended	N-amended	
9	85	78	81^{ab}
12	90	83	87^{b}
21	83	65	74^{ab}
24	77	61	69^{ab}
33	53	39	46^{ab}
36	52	46	49^{ab}
45	28	32	30^{a}
Treatment $\bar{X}$	67^{a}	58^{a}	

---

[1] Data analyzed by two-way ANOVA (MSE = 1032.0) and Scheffé multiple contrasts for pairwise comparisons. Values in the time $\bar{X}$ column or treatment $\bar{X}$ row followed by the same letter(s) do not differ significantly ($p \leq 0.05$).

Table 26. Decomposition (% dry wt remaining) of N-amended and unamended fir dowel at the undisturbed and disturbed prairie grassland sites[1].

Time (mo)	% dry wt remaining			
	Undisturbed site		Disturbed site	
	Unamended	N-amended	Unamended	N-amended
6	99	95	100	96
12	99	94	100	98
20	99	97	100	98
24	98	94	99	97
32	98	95	100	97
44	100	95	100	97

[1] Data for the woody substrates were not analyzed due to lack of variation.

Table 27. Decomposition of unamended and N-amended fir dowel and spruce wood blocks at the undisturbed and disturbed subalpine sites[1].

Time (mo)	% dry wt remaining					
	Undisturbed site			Disturbed site		
	Unamended fir	Amended fir	Spruce	Unamended fir	Amended fir	Spruce
9	100	98	98	99	97	93
12	96	93	98	100	98	98
21	100	96	98	99	98	98
24	100	97	96	99	97	96
33	100	99	97	100	98	100
36	100	96	97	100	92	93
45	99	97	-	-	-	-

---

[1] Data not analyzed due to lack of variation.

with no differences observed between undisturbed and disturbed plots (Table 27).

3.1.7 Decay Rates of Native Leaf Litter and Woody Residues

The rate of decay of the native woody substrates at all three sites (i.e. branchwood of *Artemisia cana*, *Abies lasiocarpa* and branches and cones of *Pinus banksiana* at the grassland, subalpine and oil sands sites respectively) was observed to be extremely slow with 80-90% wt remaining after 33 (oil sands site) or 45 mo incubation in the field (Tables 28-30, Figs. 8-10). The decomposition of *Agropyron* grass litter and fir needles was more rapid than that of the woody substrates (Tables 28 and 29) with grass litter at the grassland site decomposing faster than needles at the subalpine site. Over the first 24 mo of the study, jack pine needles placed in bags tended to lose wt more rapidly than those strung on thread (Table 30, Fig. 10). Pine and fir needle litter demonstrated short term gains in wt; the worst example being that of bagged pine needles which had significantly more wt remaining after 33 mo than 24 mo (Table 30). Filter paper decomposed at a faster rate than pine needle litter at the oil sands site (Table 30, Fig. 10). However, at the grassland site there was 72% wt remaining of the unamended filters after 44 mo in the field (Table 22) compared with 40% wt remaining of the *Agropyron* grass litter after the same time interval (Table 28). There was very little difference between the wt remaining of unamended filters and fir needles after 45 mo incubation at the subalpine site (Tables 24, 29).

3.1.8 Effect of Revegetation on Microbial Respiration and Biomass C at the Subalpine Site

Disturbance through mining caused a significant increase in the pH from 4.5 in the undisturbed organic soil to 5.9 in the stockpiled regolith (Table 31). As reclamation progressed the pH increased significantly to 7.3 in a site revegetated for 7 yr. Organic matter content was shown to be significantly lower in the stockpiled regolith and soil from areas in the early stages of

Table 28. Decomposition of Agropyron sp. grass litter and Artemisia cana stems at the prairie grassland undisturbed site[1].

	% dry wt remaining	
Time (mo)	Grass litter	Woody stems
6	89^{d}	95^{a}
12	61^{c}	91^{a}
20	53^{bc}	93^{a}
24	41^{ab}	90^{a}
32	45^{ab}	88^{a}
44	40^{a}	88^{a}
MSE	27.8	.0156

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[1] Data analyzed by a one-way ANOVA and Scheffé multiple contrasts for pairwise comparisons. Values in each column followed by the same letter(s) do not differ significantly ($p \leq 0.05$). Data for woody stems required a 2 arcsin $\sqrt{p}$ transformation; means are, therefore, geometric.

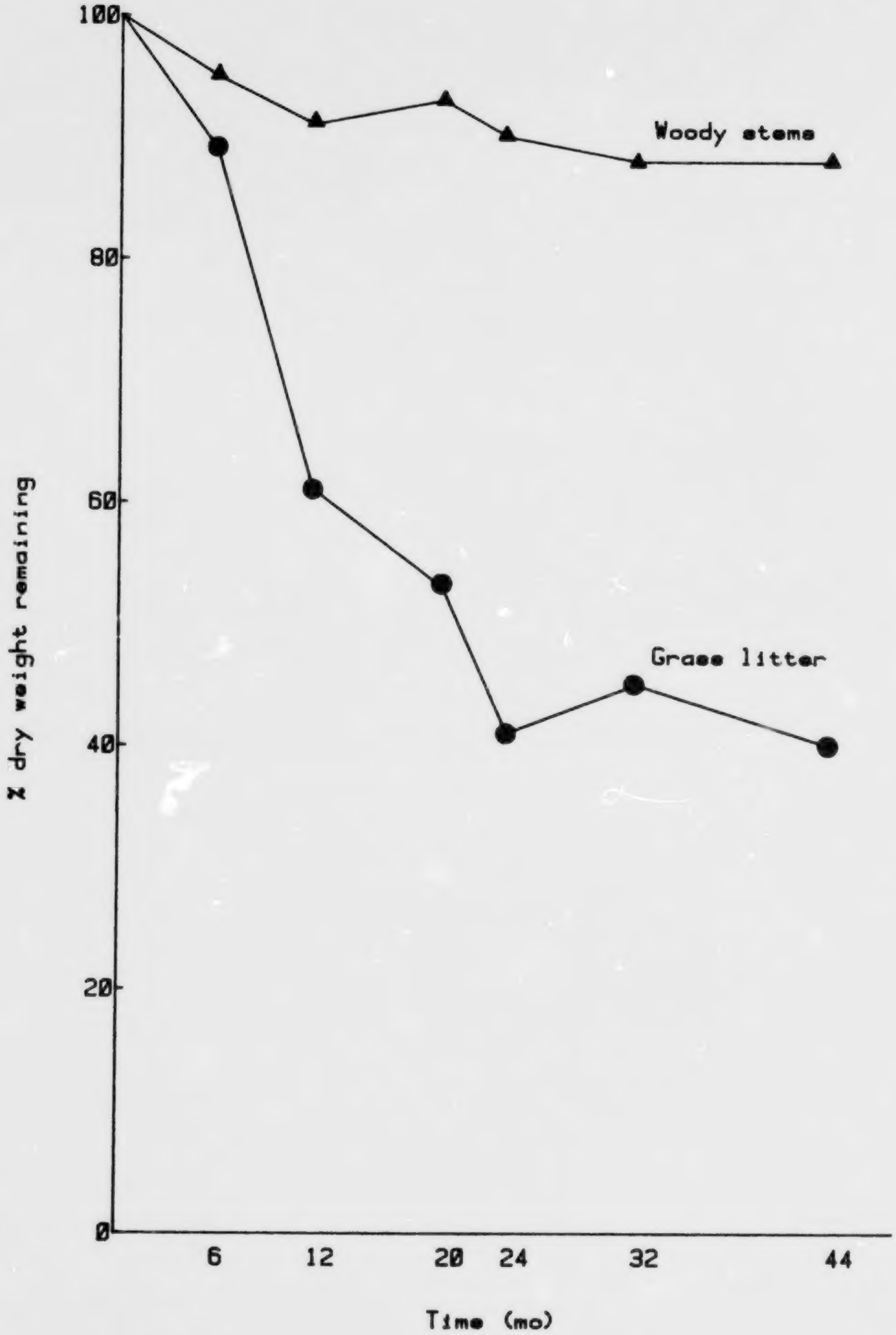

Fig. 8 Decomposition of *Agropyron sp.* grass litter and *Artemisia cana* stems at the grassland undisturbed site.

Table 29. Decomposition of Abies lasiocarpa needles and branch wood at the subalpine undisturbed site.

Time (mo)	% dry wt remaining Needles[1]	Branch wood
9	95^{b}	98^{b}
12	83^{ab}	95^{b}
21	82^{ab}	95^{b}
24	77^{ab}	93^{b}
33	78^{ab}	98^{b}
36	72^{ab}	96^{b}
45	67^{a}	84^{a}
MSE	.0353	.0438

[1] Data analyzed by one-way ANOVA after 2 arcsin $\sqrt{p}$ transformation. Significant differences determined by Scheffé multiple contrasts for pairwise comparisons. For the branch wood data, the mean of the first six sample times was compared to the mean of the last sample time to determine if a difference existed. Values in each column followed by same letter(s) do not differ significantly ($p \leq 0.05$). Means are geometric. MSE = mean square error.

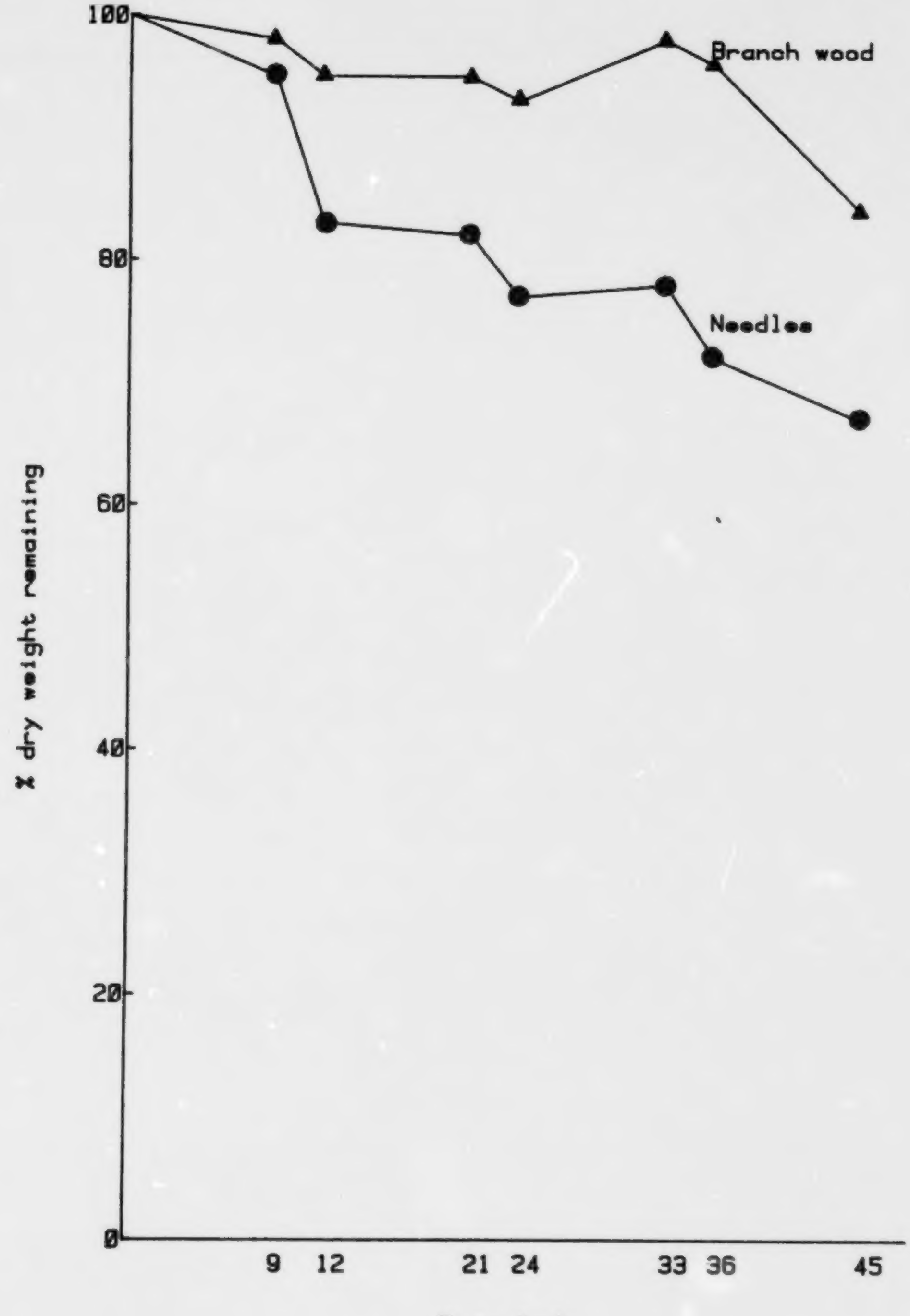

Fig. 9 Decomposition of *Abies lasiocarpa* needles and branch wood at the subalpine undisturbed site.

Table 30. Decomposition of various substrates at the undisturbed oil sands site[1].

Substrate	% dry wt remaining					MSE
	9 mo	12 mo	21 mo	24 mo	33 mo	
Filter paper[2]	88^{c}	50^{b}	19^{ab}	4^{a}	6^{a}	.13
Fir dowel	98	96	99	97	97	.54
Jack pine needles (bag)	88^{b}	78^{ab}	77^{ab}	68^{a}	81^{b}	30.96
Jack pine needles (strung)	94^{a}	84^{a}	88^{a}	88^{a}	-	38.52
Jack pine branch wood[2]	95^{b}	92^{ab}	95^{b}	88^{ab}	85^{a}	.046
Jack pine cones[2]	99^{b}	96^{ab}	97^{ab}	94^{a}	92^{a}	.015

---

[1] Data analyzed by one-way ANOVA and Scheffé multiple contrasts for pairwise comparisons. Values in each row followed by same letter(s) do not differ significantly ($p \leq 0.05$). MSE = mean square error.

[1] Data required 2 arcsin $\sqrt{p}$ transformation. Means are geometric.

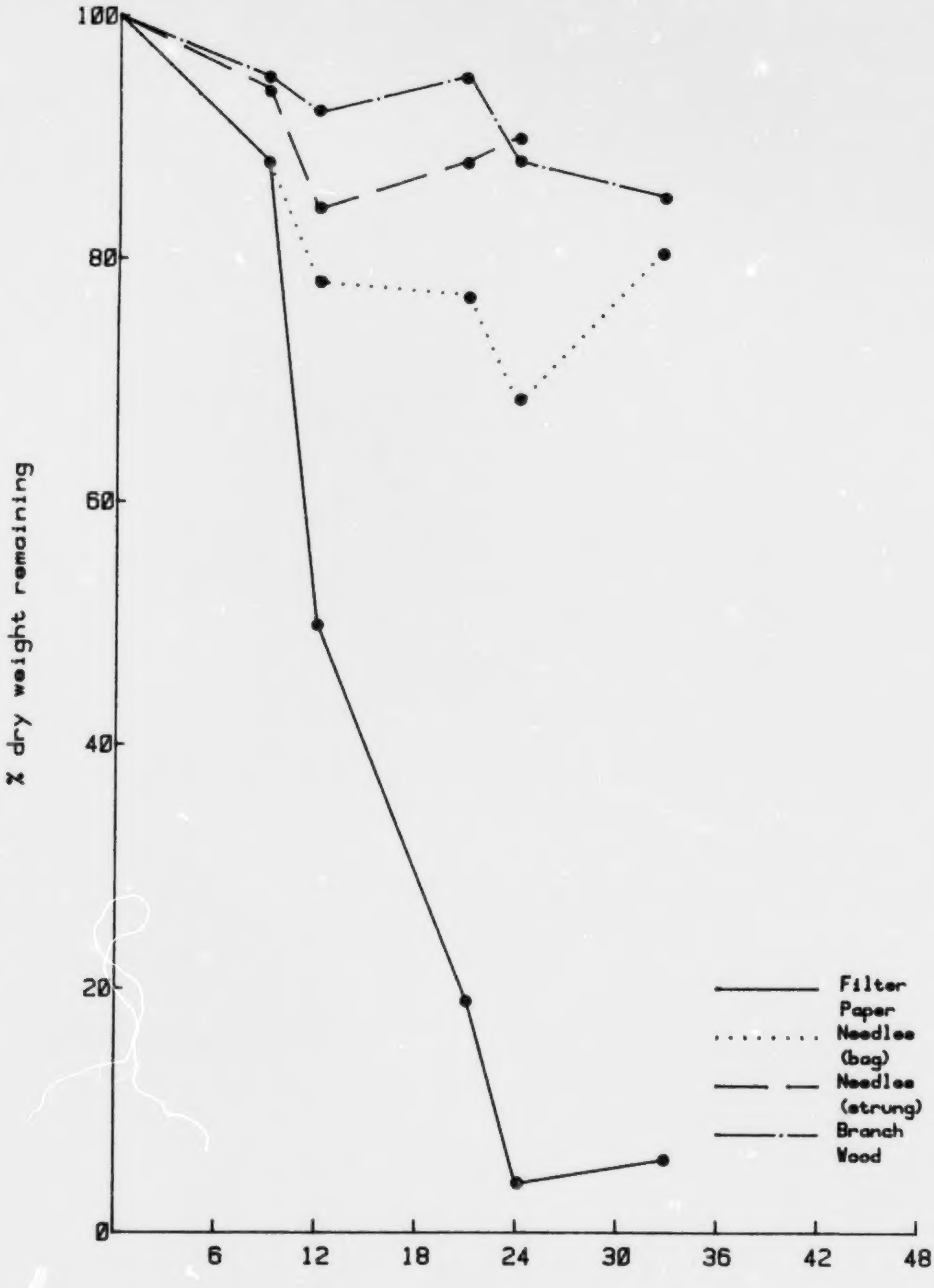

Fig. 10 Decomposition of various substrates at the undisturbed oil sands site.

Table 31. Microbial respiration and biomass in soil from an undisturbed subalpine forest (spruce/fir) and from adjacent areas in various stages of reclamation[1].

Soil description	pH (.01 M $CaCl_2$)	Organic matter	Moisture (% wet wt)	Basal respiration ($\mu l\ CO_2 \uparrow\ 100g^{-1}$ dwt hr^{-1})	Microbial biomass C (mg C $100g^{-1}$ dwt)
Undisturbed organic (0-10 cm)	4.5^{a}	85.8^{c}	74.0 (1.7)[2]	6946^{d}[3]	1151.7^{d}
Undisturbed mineral (10-20 cm)	4.7^{a}	5.9^{a}	24.3 (9.1)	86^{a}	22.4^{ab}
Mineral (0-10 cm) (revegetated for 7 yr)	7.3^{d}	18.8^{b}	31.3 (11.4)	1073^{c}	110.7^{c}
Mineral (0-10 cm) (revegetated for 6 yr)	7.5^{d}	6.7^{a}	15.2 (1.7)	436^{b}	45.6^{bc}
Mineral (0-10 cm) (revegetated for 2 yr)	6.8^{c}	2.3^{a}	12.2 (0.4)	115^{a}	25.6^{ab}
Stockpiled regolith (0-10 cm)	5.9^{b}	3.7^{a}	13.1 (0.5)	50^{a}	13.2^{a}
MSE	.04	14.2		6137	0.15

---

[1] Data analyzed by one-way ANOVA and Scheffé multiple contrasts. Values in each row followed by same letter(s) do not differ significantly ($p \leq .05$). MSE = mean square error.

[2] ( ) = standard deviation.

[3] Data required a ln X transformation. Values are geometric means.

revegetation than in the undisturbed organic soil. However, the organic matter level in the regolith was found to be comparable to that in the undisturbed mineral soil. The site revegetated for 7 yr had significantly more organic matter than the other disturbed and revegetated sites. Basal respiration (CO_2 efflux) and microbial biomass C were significantly greater in the undisturbed organic soil than any of the other soils. As reclamation (and soil pedogenesis) progressed, both basal respiration and microbial biomass C demonstrated an increasing trend. Interestingly, CO_2 efflux and microbial biomass C in the stockpiled regolith was not significantly different from that measured in the undisturbed mineral soil. The microbial biomass C measured in the various soils demonstrated a significant relationship to the soil organic matter content (Fig. 11).

3.2. TANK STUDY

3.2.1 Microbial Characteristics of Individual Amendments

Some of the chemical and physical characteristics of the amendments have been presented in Table 6a, Visser et al. (1984).

Data on the microbial parameters determined for each of the amendments are given in Tables 32 and 33. The bacteria were most numerous in the sewage sludge and the peat, while actinomycetes were only recorded from the peat. These results do not mean that there were no actinomycetes present in the sewage sludge, fertilizer and gypsum. It is possible that if lower soil dilutions (i.e. < 10^{-4}) had been plated out, actinomycetes would have been isolated from these amendments also. Hyphal length (both total and that with cell contents) was significantly greater in the sewage sludge and peat than in the fertilizer and gypsum.

The bacterial numbers and hyphal length results supported the microbial activity measured in three of the amendments, i.e. peat and sewage sludge with their high densities of bacteria and fungal hyphae were also highly active in terms of O_2 consumption (Table 34). ATP levels in the sewage sludge were also very high.

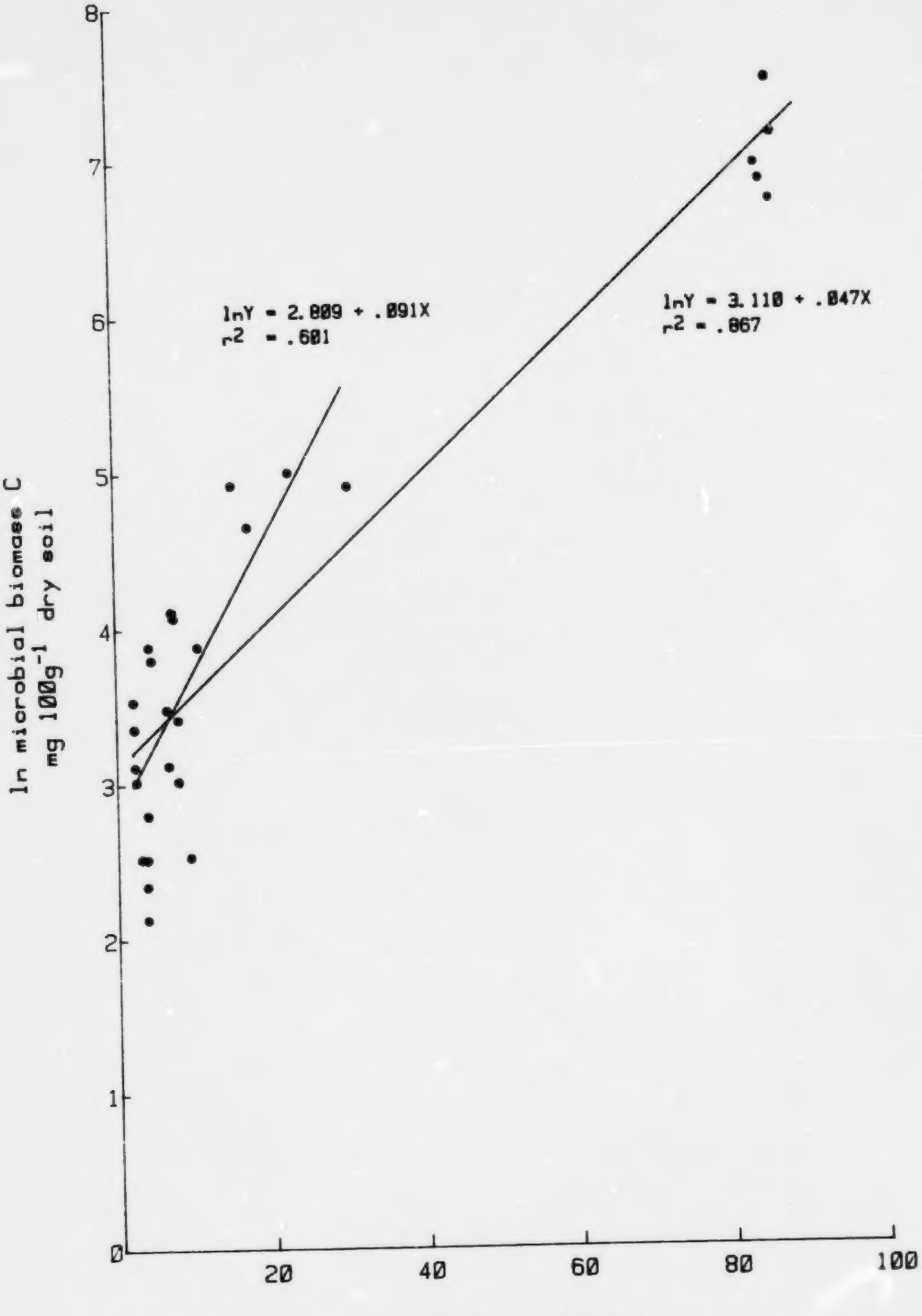

Fig. 11 Relationship between microbial biomass C and percent organic matter for subalpine soil in various stages of reclamation.

Table 32. Bacterial and actinomycete numbers in amendments prior to application to tank soils. Values are means of 3 replicates plus standard deviations.

		Amendment			
		Sewage sludge	Fertilizer	Gypsum	Peat
Bacteria					
(no. x 10^5 g^{-1} dwt soil)	$\bar{X}$	42320	1.3	0.11	6560
	SD	7240	-	.02	4860
Actinomycetes					
(no. x 10^4 g^{-1} dwt soil)	$\bar{X}$	$< 10^4$	$< 10^4$	$< 10^4$	1506
	SD	-	-	-	198

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[1] No SD was calculated for bacteria from the fertilizer since 2 of the 3 replicates had $<10^5$ bacteria g^{-1} dwt fertilizer. Dilutions of $<10^5$ were not plated out.

Table 33. Hyphal lengths in amendments prior to application to tank soils[1].

Hyphal category	Amendment				
(m g^{-1} dwt amendment)	Sewage Sludge	Fertilizer	Gypsum	Peat	MSE
Total hyphal length	2553^c	32^a	79^b	2275^c	0.041
Total hyphal length with cell contents	401^b	22^a	45^a	345^b	0.208

[1] Data in each hyphal category were ln Y transformed and analyzed with a one-way ANOVA and Scheffé multiple contrasts for pairwise comparisons. Values in each row are geometric and where followed by the same letter do not differ significantly ($p \leq 0.05$). MSE = mean square error.

Table 34. Microbial activity ($O_2\downarrow$) and ATP levels in amendments prior to application to tank soils. Values are means (n = 3) ± SE. Fertilizer was not tested.

Amendment	$O_2\downarrow$ (µl 100g^{-1} dwt hr^{-1})	ATP (µg 100 g^{-1})
Gypsum	28 ± 6	ND[1]
Peat	1106 ± 293	.10 ± .10
Sewage sludge	6269 ± 499	375.5 ± 137.9

[1] ND = not determined.

3.2.2. Prairie Grassland Minespoil - Effects of Amendation and Plant Growth on Microbial Development

3.2.2.1 Microbial numbers. The ATP measurements made approximately 0.5 mo after amendation were highly variable with no ATP recorded from the control, untreated soil, and very high (and variable) levels in the topsoil treatment (Table 35). In general, ATP levels in the subsurface soil were so low that they were not measureable.

In the surface soil, bacterial counts were significantly higher in the sewage sludge treatment than in the gypsum and topsoil treated and the untreated minespoil (Table 36). However, in the subsurface soil (5-15 cm depth; 15-25 cm in topsoil treatment) amendation and plant growth did not significantly influence the numbers of bacteria. Overall, bacterial numbers did not differ significantly between the two sampling times at either of the soil depths in any of the treatments (Table 36). The data, particularly that from the sewage sludge treatment, was highly variable.

The actinomycete numbers recorded in the prairie minespoil plots, 0.5 and 15 mo after amendation, are presented in Table 37. These data indicate that:

i) in the surface soil, there were no immediate effects of amendation on the actinomycete counts. However, 15 mo after amendation actinomycete numbers were significantly lower in the gypsum treated spoil than in the untreated spoil.

ii) at the deeper soil depth, gypsum application resulted in fewer actinomycetes than that recorded in the untreated control spoil.

iii) in general, in the surface spoil layer, amendation had no significant influence on actinomycete numbers over the two sampling times, but in the subsurface soil actinomycetes were more numerous 15 mo after amendation than 0.5 mo after amendation.

3.2.2.2 Litter input and loss on ignition. Growing fall rye in the sewage sludge and topsoil amended spoil resulted in significantly more grass litter input than growing fall rye in the untreated and

Table 35. ATP levels in grassland spoil 0.5 mo after amendation. Values are $\bar{X} \pm$ SD.

Amendment	Soil depth (cm)	ATP ($\mu g\ 100g^{-1}$ dry soil)
Control	0-5	0
	5-15	0
Gypsum	0-5	1.1 ± 1.9
	5-15	0
Sewage sludge	0-5	0.13 ± 0.12
	5-15	0.16 ± 0.29
Topsoil	0-5	152.03 ± 162.93
	15-25	0

Table 36. Effect of amendation and time on bacterial numbers in the prairie grassland tank plots[1]. Plots were planted with Fall Rye.

Amendment	Depth (cm)	No. of bacteria x $10^7 g^{-1}$ dwt soil Time after amendation (mo) 0.5	 15	Amendment $\overline{X}$
Control	0-5	5.49	3.92	4.64^a
Gypsum		4.38	15.32	8.19^a
Sewage		18.64	136.65	50.47^b
Topsoil		11.31	5.58	7.95^a
Time $\overline{X}$		8.44^a	14.63^a	
Control	5-15	4.44	3.54	3.99^a
Gypsum		4.52	1.64	3.08^a
Sewage		7.46	9.34	8.40^a
Topsoil	15-25	4.43	2.90	3.67^a
Time $\overline{X}$		5.21^a	4.36^a	

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[1] Data analyzed by a two-way ANOVA (MSE = .845 and 11.80 for 0-5 cm and 5-15 cm depths respectively) after ln Y transformation. Values in the amendment $\overline{X}$ column or time $\overline{X}$ row followed by the same letter do not differ significantly ($p \leq 0.05$) as determined by Scheffé multiple contrasts for pairwise comparisons. All values are geometric.

Table 37. Effect of amendation and time on actinomycete numbers in the prairie grassland tank plots[1]. Plots were planted with Fall Rye.

Amendment	Depth (cm)	No. of actinomycetes x $10^5 g^{-1}$ dwt soil Time after amendation (mo)		Amendment $\overline{X}$
		0.5	15	
Control	0-5	69.22^{ab}	278.13^{b}	
Gypsum		58.18^{ab}	50.16^{a}	
Sewage		43.84^{a}	112.92^{ab}	
Topsoil		109.27^{ab}	67.26^{ab}	
Control	5-15	90.09	303.47	165.35^{b}
Gypsum		18.93	37.73	26.73^{a}
Sewage		105.72	95.38	100.42^{ab}
Topsoil	15-25	16.58	75.67	35.42^{ab}
Time $\overline{X}$		41.58^{a}	95.35^{b}	

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[1] Data analyzed by a two-way ANOVA (MSE = .204 and .855 for 0-5 cm and 5-15 cm depths respectively) after ln Y transformation. A significant interaction was detected for the 0-5 cm data, hence Scheffé multiple contrasts for pairwise comparisons were applied to all values. Values followed by the same letter(s) do not differ significantly ($p \leq 0.05$). For the 5-15 cm data values in the amendment $\overline{X}$ column or time $\overline{X}$ row followed by the same letter(s) do not differ significantly ($p \leq 0.05$) as determined by Scheffé multiple contrasts for pairwise comparisons. All values are geometric.

gypsum amended spoil (Table 38). After 27 mo growth (i.e. after three growing seasons) roots in the 0-5 cm soil weighed more in the topsoil than in the other treatments; however in the deeper 5-15 cm soil root wts were greater in the sewage sludge amended spoil than in the control and gypsum treated spoil (Table 38).

Although amendation significantly influenced fall rye litter input through primary production, loss on ignition measurements in surface soil over the three sampling times were not significantly affected (Table 39). Sewage sludge appeared to significantly increase the organic matter levels of the 0-5 cm soil, but no differences in amounts of organic matter were detected in the subsurface soil in any of the treatments. In the 5-15 cm deep soil, loss on ignition values measured 15 mo after amendation were greater than those measured 27 mo after treatment. The results obtained for % loss on ignition should be viewed with caution since coal contamination of the minespoil would no doubt lead to an overestimate of the amount of "biologically available" organic carbon. Intuitively, the sewage sludge and topsoil treated spoil would have higher levels of active carbon than the gypsum treated and control spoil.

3.2.2.3 Respiratory activity and microbial biomass. The respiratory activity and microbial biomass C measured in the variously treated spoil over the term of the study are summarized in Tables 40, 41 and 42. Fig. 12 presents the microbial biomass C measurements in comparison with the shoot production by fall rye over the three growing seasons. The main features emerging from these data are as follows:

i) CO_2 evolution from the 0-5 cm deep soil over the three sampling times demonstrated a trend with the topsoil treatment being the most active, followed by sewage sludge, gypsum and the untreated spoil (Table 40). In this surface soil, microbial activity was significantly greater in the samples removed 15 mo after amendation. At this time primary production was also at its peak (Fig. 12).

Table 38. Fall rye litter (dead leaves and stems) input and root wts for the amended grassland spoil plots[1].

Time after planting (mo)	Plant parameter	Soil depth (cm)	Litter or root wt (g dry m^{-2})				
			Control	Gypsum	Sewage sludge	Topsoil	MSE
27	Litter	0-5	53.0^{a}	59.5^{a}	245.7^{b}	181.1^{ab}	2589.1
	Roots	0-5	3.8^{a}	5.5^{a}	7.7^{a}	53.6^{b}	0.3
	Roots	5-15 (15-25 in topsoil)	3.2^{ab}	2.6^{a}	9.1^{b}	6.3^{ab}	5.3

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[1] Data analyzed by one-way ANOVA and Scheffé multiple contrasts for pairwise comparisons. Values in each row followed by the same letter(s) do not differ significantly ($p \leq 0.05$). The 0-5 cm root data required a ln Y transformation, hence the means for this data are geometric.

Table 39. Effect of time on % loss on ignition of amended grassland spoil planted with fall rye[1,2].

Soil depth (cm)	Time after amendation (mo)	Amendment				Time $\overline{X}$
		Control	Gypsum	Sewage sludge	Topsoil	
0-5	0.5	6.11	5.79	8.57	7.56	7.01^a
	15.0	7.39	7.49	7.58	6.87	7.33^a
	27.0	5.67	6.08	8.55	6.26	6.64^a
	Amendment $\overline{X}$	6.39^a	6.45^a	8.23^b	6.90^a	
5-15 (15-25 in topsoil)	0.5	6.35	7.31	7.28	5.90	6.71
	15.0	7.22	8.15	7.40	6.79	7.39^b
	27.0	5.62	5.44	5.80	5.92	5.69^a
	Amendment $\overline{X}$	6.40^a	6.97^a	6.82^a	6.20^a	

[1] Data analyzed by two-way ANOVA (MSE = 0.79 and 1.04 for 0-5 and 5-15 cm depths respectively). No interactions were encountered, hence Scheffé multiple contrasts for pairwise comparisons were applied to time and amendment means. Values in the time $\overline{X}$ columns or amendment $\overline{X}$ rows followed by the same letters do not differ significantly ($p \leq 0.05$).

[2] Loss on ignition values for all samples, but the topsoil (0-5 cm) are overestimates due to coal particle contamination.

Table 40. Development of microbial activity ($\mu l\ CO_2 \uparrow 100\ g^{-1}$ dwt soil hr^{-1}) in amended grassland spoil planted with fall rye[1].

Soil depth (cm)	Time after amendation (mo)	Amendment				Time $\overline{X}$
		Control	Gypsum	Sewage sludge	Topsoil	
0-5	0.5	230	200	380	630	360^a
	15.0	430	760	800	700	670^b
	27.0	230	420	390	570	400^a
	Amendment $\overline{X}$	290^a	460^{ab}	520^{bc}	630^c	
5-15 (15-25 in topsoil)	0.5	560^{ab}	530^{ab}	970^{ab}	820^{ab}	
	15.0	620^{ab}	1090^b	870^{ab}	660^{ab}	
	27.0	360^a	400^a	440^a	420^a	

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[1] Data analyzed by two-way ANOVA (MSE = 15 and 25 for 0-5 and 5-15 cm depths respectively). Scheffé multiple contrasts for pairwise comparisons were applied to the time and amendment means in the 0-5 cm data set (no significant interaction was observed) and the individual means in the 5-15 cm data set (a significant interaction was observed). For the 0-5 cm data, values in the time $\overline{X}$ column or amendment $\overline{X}$ row followed by the same letter(s) do not differ significantly ($p \leq 0.05$). For the 5-15 cm data, values followed by the same letters do not differ significantly ($p \leq 0.05$).

Table 41. Development of microbial biomass (mg C 100 g^{-1} dwt soil) in amended grassland spoil planted with fall rye[1].

Soil depth (cm)	Time after amendation (mo)	Amendment			
		Control	Gypsum	Sewage sludge	Topsoil
0-5	0.5	11.72^{a}	8.11^{a}	15.72^{ab}	60.37^{bcd}
	15.0	31.89^{abcd}	77.61^{cde}	90.63^{cde}	128.70^{de}
	27.0	23.80^{abc}	61.23^{bcde}	76.64^{cde}	151.66^{e}
5-15 (15-25 in topsoil)	0.5	33.46^{a}	49.36^{a}	66.84^{ab}	61.49^{ab}
	15.0	61.95^{ab}	52.85^{a}	55.76^{a}	51.21^{a}
	27.0	45.82^{a}	51.12^{a}	51.56^{a}	121.16^{b}

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[1] Data analyzed by two-way ANOVA (MSE = .125 and 233.48 for 0-5 and 5-15 cm depths respectively). Significant interactions were encountered, hence Scheffé multiple contrasts for pairwise comparisons were applied to individual means. Values followed by the same letter(s) do not differ significantly ($p \leq 0.05$). The 0-5 cm data required a ln transformation so all means are geometric.

Table 42. The effect of sampling depth on microbial activity ($CO_2\uparrow$) and microbial biomass C in the amended grassland spoil, planted with fall rye[1].

Parameter	Time after amendation (mo)	Amendment			
		Control	Gypsum	Sewage sludge	Topsoil
Microbial activity ($CO_2\uparrow$)	0.5	**	**	**	**
	15.0	**	**	NS	NS
	27.0	NS	NS	NS	NS
Microbial biomass C	0.5	NS	**	**	NS
	15.0	NS	NS	**	*
	27.0	NS	NS	**	*

---

[1] As a result of dependence between the 0-5 and 5-15 cm data, the effect of depth was determined by subtracting the 5-15 cm values from the 0-5 cm values an analyzing the resulting data using the model for the two-way ANOVA (i.e. microbial biomass C = $\bar{\mu}$ .. + (amendment effect) + (time effect) + error). The null hypothesis was that each treatment mean = 0 (i.e. no difference between depths). This method allows examination of each treatment in the event that not all treatments show an effect of depth.

NS - depths not significantly different ($p \leq 0.05$).

* - 0-5 cm significantly greater than 5-15 $\bar{c}$m (15-25 in topsoil) ($p \leq 0.05$).

** - 5-15 cm significantly greater than 0-5 cm ($p \leq 0.05$).

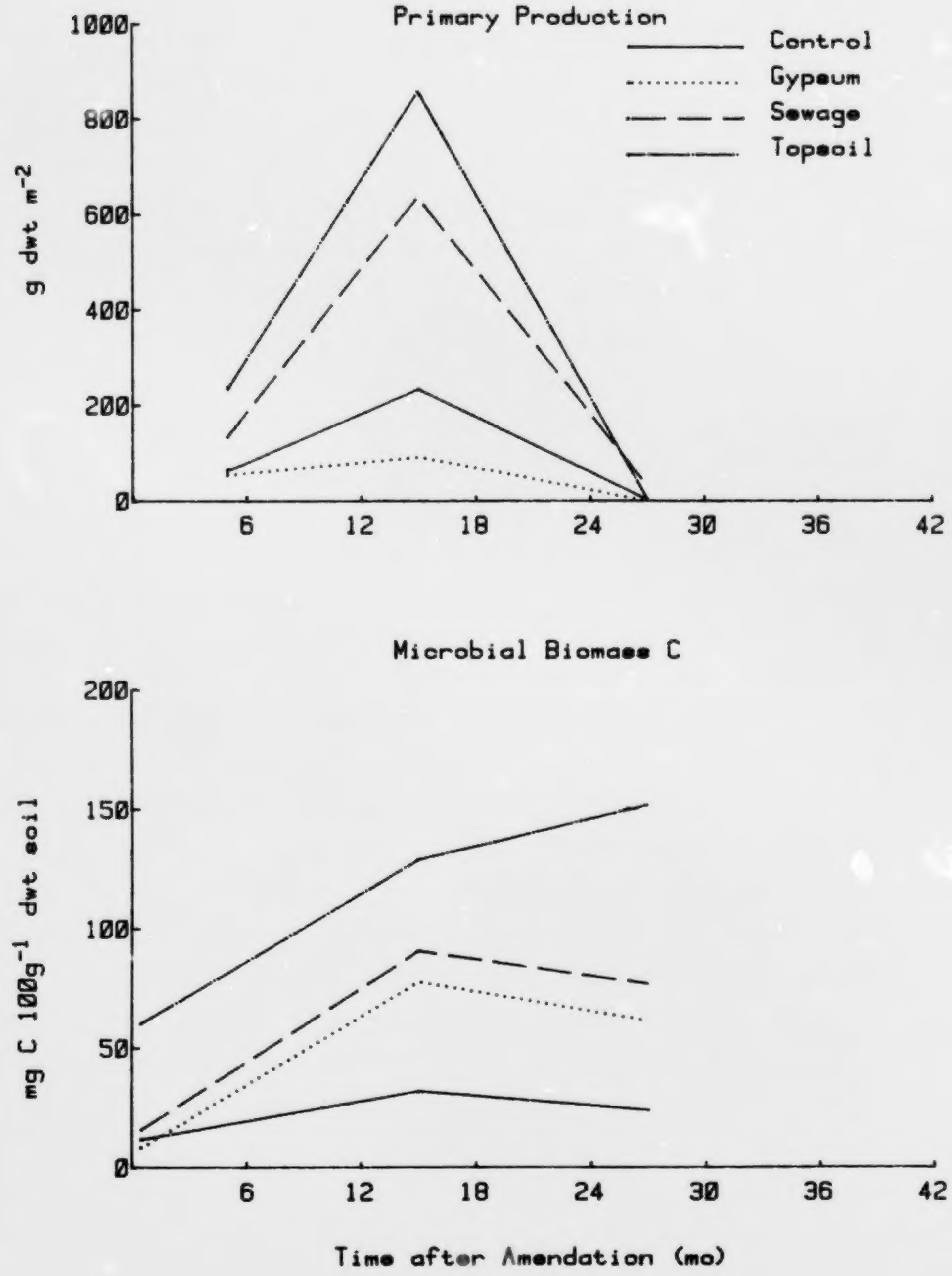

Fig. 12 Primary production and the development of microbial biomass C in amended grassland spoil planted with fall rye.

ii) unlike the surface soil, amendation and plant growth had very little influence on the respiratory activity in the subsurface soil (Table 40). Respiration measured 0.5 mo after amendation was significantly higher in the subsurface than the surface soil; however, at 15 mo after amendation no significant differences were detected between CO_2 efflux from soil at either depth (Table 41).

iii) immediately after amendation, microbial biomass C in 0-5 cm soil was highest in the topsoil treatment followed by sewage sludge, gypsum and control treatments (Table 41, Fig. 12). Fifteen months after amendation, the microbial biomass had increased significantly in the gypsum and sewage sludge treated spoil. The biomass in the topsoil treatment also increased, but this increase was not significant until 27 mo after amendation. The microbial biomass C demonstrated its greatest increase after the second growing season (15 mo) when fall rye was also most productive (Fig. 12).

iv) microbial biomass was less affected by amendation and subsequent plant growth in the 5-15 cm deep soil than the 0-5 cm deep soil (Table 41). However, the spoil beneath the topsoil treatment (i.e. 15-25 cm deep soil) contained more microbial biomass C 27 mo after amendation than at the previous two sampling times.

v) no soil depth effects on microbial biomass C were detected in the untreated plots, while the 5-15 cm deep sewage sludge treated spoil contained more microbial biomass C than the surface soil (Table 42). The biomass tended to be higher in the topsoil than in the subsurface spoil material (Table 42).

Product moment correlation coefficients calculated between % loss on ignition, grass litter input, grass root wt, microbial activity and microbial biomass C demonstrated that fall rye root wts and microbial activity and biomass C were highly correlated (Table 43). Microbial activity and microbial biomass C, % loss on ignition and litter input were also strongly related. Soil respiratory activity and microbial biomass C demonstrated a positive correlation with grass litter input, but not as strong a correlation as obtained with grass root wts.

Table 43. Product moment correlation coefficients for loss on ignition, litter input, root wt, $CO_2 \uparrow$ and microbial biomass C data collected 27 mo after amendation of the grassland spoil (fall rye plots).

	% loss on ignition	Grass litter input	Grass root wt	Microbial activity ($CO_2\uparrow$)	Microbial biomass C
% loss on ignition	1	.829	-.042	.230	.398
Grass litter input		1.00	.417	.526	.607
Grass root wt			1.00	.768	.772
Microbial activity ($CO_2\uparrow$)				1.00	.873
Microbial biomass C					1.00

3.2.2.4 Decomposition of standard substrates. The decomposition potential of the grassland minespoil as measured by wt loss of cellulose filter paper after 1 yr incubation in the field was greater if the spoil had been amended with topsoil than if it had been left untreated or treated with gypsum (Table 44). This was true in amended spoil planted with either fall rye or rambler alfalfa. After 2 yrs incubation in the variously treated plots planted with fall rye there were no significant differences in wt loss of the filters amongst the four treatments. In the fall rye plots, % wt remaining in the untreated plots was significantly less after 24 mo incubation than after 12 mo incubation; this was not the case for the treated plots where no significant differences in wt loss were observed between the two sampling times. In plots planted with rambler alfalfa, there was a significant decrease in % dwt filter remaining between 12 mo and 24 mo incubation regardless of amendment (Table 44). Over the short term (12 mo) filters in the sewage sludge and topsoil treated plots tended to degrade more rapidly if the plots had been planted with fall rye rather than rambler alfalfa, but after 24 mo incubation no differences were measured between the two vegetation types.

In contrast to the filter paper, fir wood dowel demonstrated negligible wt loss over the 24 mo incubation regardless of treatment or vegetation type (Table 45).

Changes in the decomposition potential over time of the variously amended spoil were tested by measuring wt loss of filters placed in the plots 4 mo and 28 mo after planting fall rye. The results obtained are presented in Table 46 and Fig. 13 and show that the decomposition potential of the gypsum and sewage sludge treated and untreated spoil had not changed significantly over time, i.e. the growth of fall rye had not improved the decomposing ability of the spoil over the 40 mo term of the study. Filters placed in the topsoil treated plots lost less wt when placed in the plots 28 mo after planting than 4 mo after planting fall rye. Four mo after planting fall rye, the decomposition potential of the topsoil and sludge treated soil was significantly greater than that of the

Table 44. Cellulose filter paper decomposition (expressed as % wt remaining) in amended grassland spoil planted with fall rye and rambler alfalfa[1]. Filters were placed in the field 4 mo after planting.

Plant type	Time in the field (mo)	Amendment				Time $\overline{X}$
		Control	Gypsum	Sewage sludge	Topsoil	
Fall rye	12	89.1^{b}	87.1^{b}	53.6^{ab}	21.2^{a}	
	24	26.6^{a}	44.1^{ab}	27.7^{a}	20.3^{a}	
Rambler alfalfa	12	99.2	85.3	82.5	58.1	81.3^{a}
	24	73.0	34.3	28.2	13.5	37.3^{b}
	Amendment $\overline{X}$	86.1^{c}	59.8^{b}	55.4^{ab}	35.8^{a}	

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[1] Data analyzed by two-way ANOVA (MSE = 163.9 and 160.0 for fall rye and rambler alfalfa respectively). A significant interaction was observed for the fall rye data, hence Scheffé multiple contrasts for pairwise comparisons were applied to individual means. Values followed by the same letter(s) do not differ significantly ($p \leq 0.05$). Scheffé multiple contrasts for pairwise comparisons were applied to rambler alfalfa time and amendment means since no interaction was observed for this data set. Values in the time $\overline{X}$ column or amendment $\overline{X}$ row followed by the same letter(s) do not differ significantly ($p \leq 0.05$).

Table 45. The % wt remaining of fir wood dowel placed in amended grassland spoil plots 4 mo after planting with fall rye and rambler alfalfa.

Plant type	Time in the field (mo)	Amendment				Time $\overline{X}$
		Control	Gypsum	Sewage sludge	Topsoil	
Fall rye	12	99.9	100	99.7	100	99.9
	24	100	100	99.9	99.8	99.9
	Amendment $\overline{X}$	99.95	100	99.8	99.9	
Rambler alfalfa	12	99.8	100	100	100	99.9
	24	100	100	100	99.9	99.95
	Amendment $\overline{X}$	99.9	100	100	99.95	

Table 46. The % wt remaining of cellulose filter paper incubated for 12 mo in amended grassland spoil plots planted with fall rye. Filters were placed in the plots 4 mo and 28 mo after planting[1].

Time after planting (mo)	Amendment			
	Control	Gypsum	Sewage sludge	Topsoil
4	89^{c}	87^{bc}	53^{ab}	21^{a}
28	89^{c}	75^{bc}	87^{bc}	79^{bc}

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[1] Data analyzed by two-way ANOVA (MSE = 266.97) and Scheffé multiple contrasts for pairwise comparisons. Values followed by the same letter(s) do not differ significantly ($p \leq 0.05$).

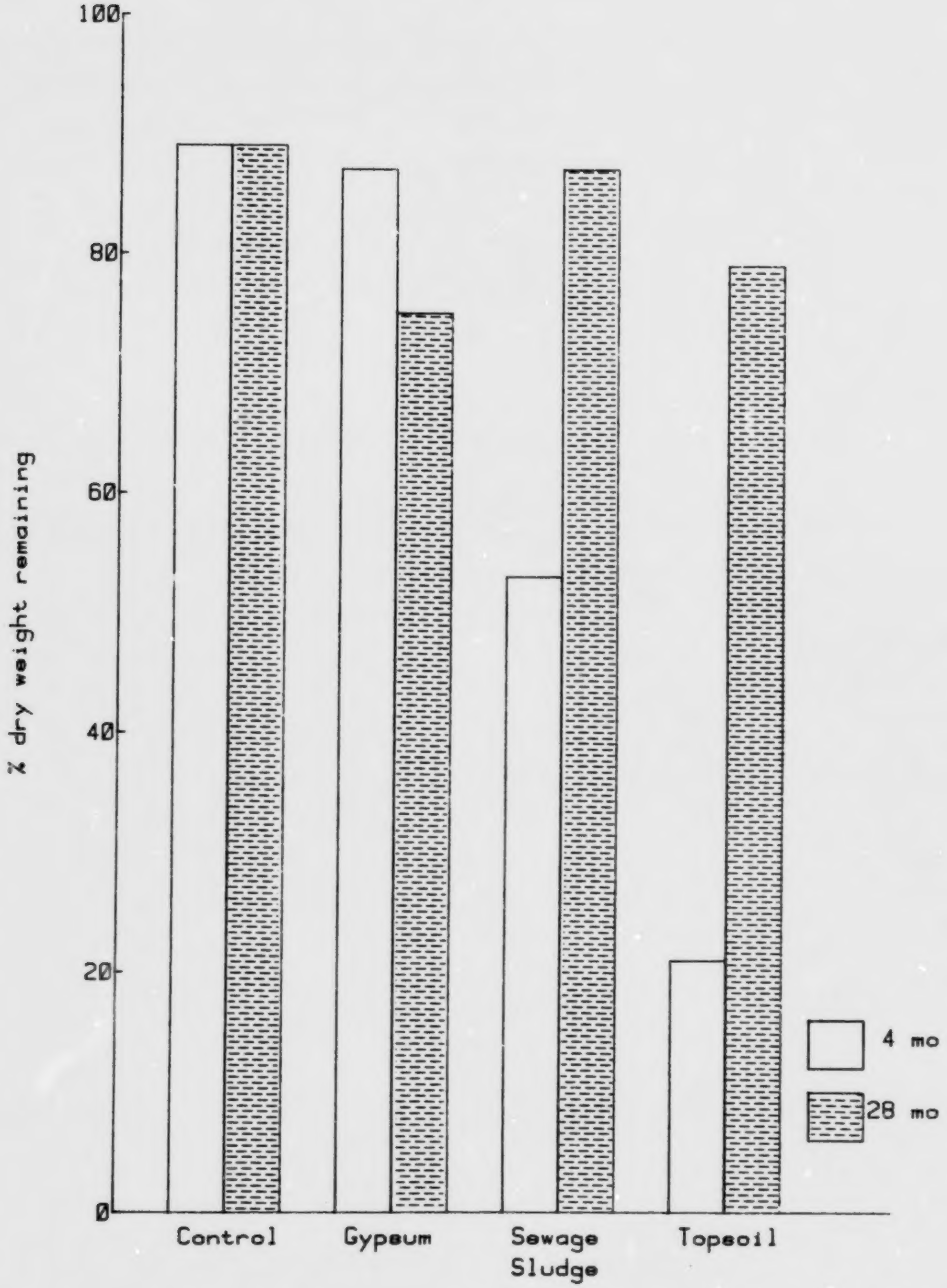

Fig. 13. The percent weight remaining of cellulose filter paper incubated for 12 months in amended grassland spoil plots planted with fall rye. Filters were placed in the plots 4 months and 28 months after planting.

control soil; however, 28 mo after planting, no significant differences in decomposition potential were detected amongst any of the treatments.

In contrast to the results obtained for the amended spoil planted with fall rye, decay of filter papers in plots planted with rambler alfalfa was faster 28 mo after planting than 4 mo after planting (Table 47). This was particularly true for the sewage sludge and topsoil treated plots, where filters decayed more rapidly than in the gypsum treated and untreated plots.

3.2.2.5 Symbiotic N_2 fixation. Data on the N_2 (C_2H_2) fixed by rambler alfalfa over the term of the study are presented in Table 48. Amendation did not significantly influence the amount of N_2 fixed by plants removed from the plots at each of the three sampling times, but measurements were highly variable particularly for plants from the sewage sludge and gypsum amended spoil. There did not appear to be any relation- ship between nodule wt and N_2 fixing capacity for alfalfa tested 14 mo after planting. At this time nodule morphology also varied amongst the treatments with roots from the topsoil treatment bearing numerous small (1 mm dia. or less) pink (indicating presence of leghaemoglobin) nodules and roots from the control, sewage sludge and gypsum treatments having large (up to 5-7 mm dia.) pink clumps of nodules. Although the effect of time on the N_2 fixing capacity of the alfalfa was not tested (since the plants may not have been in the same physiological condition after each of the three growing seasons), it appeared that N_2 fixation activity was greatest for plants collected at the conclusion of the second growing season (14 mo).

3.2.3. Subalpine Minespoil - Effects of Amendation and Plant Growth on Microbial Development

3.2.3.1 Microbial characteristics 0.5 mo and 15 mo after amendation and planting. The ATP levels in the subalpine spoil immediately after amendation were, like those in the grasslands spoil, highly

Table 47. The % dry wt remaining of cellulose filter paper after 12 mo incubation in grassland spoil plots planted with rambler alfalfa. Filters were placed in the plots at 4 mo and 28 mo after planting[1].

Time after planting (mo)	Amendment Control	 Gypsum	 Sewage sludge	 Topsoil	Time $\overline{X}$
4	100	91	87	58	88^b
28	95	91	50	45	74^a
Amendment $\overline{X}$	98^b	91^b	70^a	52^a	

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[1] Data analyzed by 2-way ANOVA (MSE = .258) after 2 arcsin $\sqrt{p}$ transformation. No interaction was observed, hence Scheffé multiple contrasts for pairwise comparisons were applied to time and treatment means. Values in the time $\overline{X}$ column or amendment $\overline{X}$ row followed by the same letter do not differ significantly ($p \leq 0.05$). Means are geometric.

Table 48. N_2 (C_2H_2) fixation capacity (nmoles g^{-1} dry root hr^{-1}) and nodule weight (mg dry $sample^{-1}$) of rambler alfalfa planted in grassland spoil tank plots[1].

	Time after planting				
Treatment	3 mo	14 mo		26 mo	
	N_2 fixed	Nodule wt	N_2 fixed	Nodule wt	N_2 fixed
Control	37.1^a	52	149.5^a	9	1.7^a
Gypsum	155.0^a	46	859.3^a	19	9.9^a
Sewage Sludge	6.5^a	30	1263.3^a	5	4.1^a
Topsoil	103.8^a	41	93.7^a	39	15.4^a
MSE	9656.81		4.11		87.47

[1] Data for each sampling time were analyzed using a one-way ANOVA after ln (y + 1) transformation. Values in each column followed by the same letter do not differ significantly ($p \leq 0.05$).

variable (Table 49). The sewage sludge treated spoil tended to have the highest ATP level while the untreated spoil had the lowest.

Both in the 0-5 cm and 5-15 cm deep subalpine spoil the bacteria were most numerous in plots treated with sewage sludge (Table 50). Bacterial numbers in the surface soil 0.5 and 15 mo after amendation were not significantly different; however, in the subsurface soil bacterial numbers were greater 15 mo after amendation than 0.5 mo after amendation.

Immediately after application of the amendments (0.5 mo), the number of actinomycetes in the surface soil was significantly higher in the peat treated plots than in the other treatments (Table 51). However, 15 mo after treatment and planting slender wheatgrass, actinomycetes in the 0-5 cm peat had decreased significantly, while those in the sewage sludge treated spoil had increased. Actinomycetes in the 5-15 cm spoil tended to be greater in the fertilized spoil than in the other treatments; also numbers increased from 0.5 to 15 mo after amendation at this depth (Table 51).

Total hyphal lengths at both sampling depths were also greatest in the peat amended spoil (Tables 52 and 53). No differences in total lengths were detected between the two sampling times, regardless of treatment. As for the total hyphal length, mycelium with cell contents in the 0-5 cm deep soil was longest in the peat; this was not the case in the subsurface soil where treatment did not significantly influence hyphal lengths with cell contents. Clamped hyphae were detected in all treatments at both depths indicating the presence of the basidiomycete group of fungi throughout the soil.

3.2.3.2 Microbial development in amended, unvegetated minespoil.

The % loss on ignition of samples from the amended, unvegetated pathways along the tank walls was measured 0.5 and 27 mo after amendation. As expected, the surface soil of the peat treated plots had the highest level of organic matter (Table 54). Loss on ignition in the 5-15 cm soil was not affected by treatment. Also, the organic

Table 49. ATP levels in subalpine spoil 0.5 mo after amendation. Values are $\overline{X} \pm$ SD.

Amendment	Soil depth (cm)	ATP (μg 100 g^{-1} dry soil)
Control	0-5	.10 ± .17
	5-15	.17 ± .29
Fertilizer	0-5	.17 ± .29
	5-15	3.26 ± 5.66
Sewage sludge	0-5	24.40 ± 21.76
	5-15	9.17 ± 15.62
Peat	0-5	5.3 ± 9.01
	15-25	0

Table 50. Effect of amendation and time on bacterial numbers in subalpine spoil tank plots[1]. Plots were planted with slender wheatgrass.

Amendment	Depth (cm)	No. of bacteria x 10^7 g^{-1} dwt soil		Amendment $\overline{X}$
		Time after amendation (mo)		
		0.5	15	
Control	0-5	13.77	16.19	14.93^a
Fertilizer		12.67	20.89	16.27^a
Sewage Sludge		304.87	155.28	217.58^c
Peat		41.15	33.91	37.36^b
Time $\overline{X}$		38.46^a	36.53^a	
Control	5-15	11.50	16.32	13.70^a
Fertilizer		9.14	18.71	13.08^a
Sewage Sludge		32.66	44.42	38.09^b
Peat	15-25	5.79	17.52	10.07^a
Time $\overline{X}$		11.87^a	22.08^b	

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[1] Data analyzed by a two-way ANOVA (MSE = .132 and .306 for 0-5 cm and 5-15 cm depths respectively) after ln Y transformation. Values in the amendment $\overline{X}$ column or time $\overline{X}$ row followed by the same letter do not differ significantly ($p \leq 0.05$). All values are geometric.

Table 51. Effect of amendation and time on actinomycete numbers in subalpine spoil tank plots[1]. Plots were planted with slender wheatgrass.

Amendment	Depth (cm)	No. of actinomycetes x 10^4 g^{-1} dwt soil Time after amendation (mo) 0.5	 15	Amendment $\overline{X}$
Control	0-5	19.86^{ab}	175.85^{bc}	
Fertilizer		34.17^{abc}	102.09^{abc}	
Sewage Sludge		13.39^{a}	334.36^{c}	
Peat		6113.14^{d}	238.48^{c}	
Control	5-15	38.66	73.73	53.39^{bc}
Fertilizer		36.03	139.42	70.87^{c}
Sewage Sludge		10.09	130.12	37.64^{ab}
Peat	15-25	15.38	50.34	27.82^{a}
Time $\overline{X}$		21.97^{a}	90.58^{b}	

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[1] Data analyzed by a two-way ANOVA (MSE = .485 and .307 for 0-5 cm and 5-15 cm depths respectively) after ln Y transformation. A significant interaction was detected for the 0-5 cm data, hence Scheffé multiple contrasts for pairwise comparisons were applied to all values. Values followed by the same letter(s) do not differ significantly ($p \leq 0.05$). For the 5-15 cm data, values in the amendment $\overline{X}$ column or time $\overline{X}$ row followed by the same letter(s) do not differ significantly ($p \leq 0.05$) as determined by Scheffé multiple contrasts for pairwise comparisons. All values are geometric.

Table 52. Hyphal lengths in 0-5 cm subalpine spoil before planting (0.5 mo) and after planting slender wheatgrass (15 mo)[1].

Hyphal category ($m\ g^{-1}$ dwt soil)	Sampling time (mo)	Amendment				Time $\overline{X}$
		Control	Fertilizer	Sewage Sludge	Peat	
Total	0.5	216^{a}	147^{a}	179^{a}	2459^{b}	
	15.0	159^{a}	201^{a}	235^{a}	1190^{b}	
Total with	0.5	42^{ab}	13^{a}	27^{a}	348^{c}	
cell contents	15.0	45^{ab}	59^{ab}	38^{ab}	161^{bc}	
Total clamped	0.5	11	4	9	205	57.2^{a}
	15.0	9	4	19	7	9.6^{a}
Amendment $\overline{X}$		10^{a}	4^{a}	14^{a}	106^{a}	

[1] Data in each hyphal category were analyzed by a two-way ANOVA after ln (Y + 1) transformation (MSE = .065, .227 and 1.925 for total, total with contents and total clamped respectively). Scheffé multiple contrasts for pairwise comparisons were applied to all values in the first two categories (as a result of significant interactions in the ANOVA). Values followed by the same letter(s) do not differ significantly ($p \leq 0.05$). In the total clamped category, values in the yearly $\overline{X}$ column or amendment $\overline{X}$ row followed by the same letter do not differ significantly ($p \leq 0.05$). All values are geometric means.

Table 53. Hyphal lengths in 5-15 cm (15-25 cm in peat) subalpine spoil before planting (0.5 mo) and after planting slender wheatgrass (15 mo)[1].

Hyphal category (m g^{-1} dwt soil)	Sampling time (mo)	Amendment				Time
		Control	Fertilizer	Sewage Sludge	Peat	$\overline{X}$
Total	0.5	160	154	159	302	194^a
	15.0	168	197	174	193	183^a
Amendment $\overline{X}$		164^a	175^a	166^a	248^b	
Total with cell contents	0.5	13	25	13	38	22^a
	15.0	45	35	35	46	40^a
Amendment $\overline{X}$		29^a	30^a	24^a	42^a	
Total clamped[2]	0.5	23	29	8	0	15^a
	15.0	0	6	10	8	6^a
Amendment $\overline{X}$		11^a	17^a	9^a	4^a	

(cont'd)

Table 53. (cont'd)

[1] Data in the total and total with cell contents categories were analyzed with a two-way ANOVA (MSE = 2.254 and 323.32 respectively) and Scheffé multiple contrasts for pairwise comparisons. Values in the time $\overline{X}$ columns or amendment $\overline{X}$ rows followed by the same letter do not differ significantly ($p \leq 0.05$).

[2] Because the 0.5 mo peat values and 15 mo control values had a mean of 0 with 0 variation, the Tukey test for additivity was applied to the averages of each treatment to test for interactions. Since there was no significant interaction, the two-way ANOVA with 1 replication/treatment (i.e. mean of 3 values/treatment) was applied (MSE = 126.2). Values in the time $\overline{X}$ column or amendment $\overline{X}$ row followed by the same letter do not differ significantly ($p \leq 0.05$).

Table 54. Effect of time on % loss on ignition of amended, unvegetated subalpine spoil (pathways)[1].

Soil depth (cm)	Time after amendation (mo)	Amendment			
		Control	Fertilizer	Sewage Sludge	Peat
0-5	0.5	6.02^{a}	7.07^{ab}	7.59^{ab}	63.62^{c}
	27.0	6.38^{a}	6.16^{a}	10.06^{b}	68.51^{c}
5-15 (15-25 in peat)	0.5	7.38^{a}	5.93^{a}	7.40^{a}	5.35^{a}
	27.0	6.03^{a}	6.26^{a}	7.30^{a}	8.19^{a}

[1] Data analyzed by two-way ANOVA (MSE = .031 and .709 for 0-5 cm and 5-15 cm depths respectively) and Scheffé multiple contrasts for pairwise comparisons. Values in each data set, followed by the same letter(s) do not differ significantly ($p \leq 0.05$). The 0-5 cm means are geometric since the data required a ln Y transformation.

matter levels were not altered over the course of the study in any of the treatments, at either of the two depths.

Microbial activity in the 0-5 cm soil was found to be highest in the peat and sewage sludge treated spoil, particularly at 14 mo after amendation (Table 55). In the deeper 5-15 cm soil, activity was also greatest in the 14 mo samples, but amendation appeared to have little effect on the CO_2 efflux.

The microbial biomass C measurements followed a similar pattern to that observed for the microbial activity measurements with the exception that there was no significant difference between microbial biomass C at 14 mo and that at 27 mo in the 0-5 cm soil (Table 56). Immediately after amendation (0.5 mo) microbial activity and biomass C in the fertilized spoil was greater at the 5-15 cm than at the 0-5 cm depth, while in the peat treated spoil, CO_2 efflux and biomass C were always higher in the peat than in the spoil (15-25 cm depth) beneath the peat (Table 57).

3.2.3.3 Microbial development in amended spoil planted with white spruce. The weight of white spruce roots sieved from soil samples used to estimate microbial activity and biomass C levels was determined 27 mo after initiation of the study. The results are presented in Table 58 and show that root wts were greatest in the 0-5 cm peat treatment. Only the peat amendment demonstrated a significant effect of depth on root wt, with root wt in the surface soil being greater than that measured in the spoil beneath the peat (Tables 58 and 59).

The % loss on ignition measured in samples from the amended white spruce plots (Table 60) were very similar to those obtained from the unvegetated pathway samples. The addition of peat to the subalpine spoil significantly increased its organic matter content whereas fertilizer and sewage sludge amendation had no significant effect. This was true only for the 0-5 cm deep soil. Organic matter levels did not change over the 27 mo term of the study in any of the treatments. The amount of organic matter in the surface soil of the

Table 55. Development of microbial activity ($\mu l\ CO_2 \uparrow 100\ g^{-1}$ dwt soil hr^{-1}) in amended, unvegetated subalpine soil (pathways)[1].

Soil depth (cm)	Time after amendation (mo)	Amendment				Time $\overline{X}$
		Control	Fertilizer	Sewage Sludge	Peat	
0-5	0.5	320	190	500	1460	460^a
	15.0	490	320	1290	4410	970^b
	27.0	220	200	260	2010	390^a
	Amendment $\overline{X}$	330^a	230^a	550^b	2350^c	
5-15 (15-25 in peat)	0.5	360	520	490	430	450^b
	15.0	560	480	1070	990	730^c
	27.0	240	260	250	360	270^a
	Amendment $\overline{X}$	360^a	400^{ab}	510^{ab}	530^b	

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[1] Data analyzed by two-way ANOVA after ln Y transformation (MSE = 115.58 and 771.30 for 0-5 and 5-15 cm depths respectively). Time and amendment means were tested by Scheffé multiple contrasts for pairwise comparisons. Values in the time $\overline{X}$ columns or amendment $\overline{X}$ rows, followed by the same letter(s) do not differ significantly ($p \leq 0.05$). All values are geometric means.

Table 56. Development of microbial biomass C (mg C 100 g^{-1} dwt soil) in amended, unvegetated subalpine spoil (pathways)[1].

Soil depth (cm)	Time after amendation (mo)	Amendment				Time $\overline{X}$
		Control	Fertilizer	Sewage Sludge	Peat	
0-5	0.5	24.08	10.34	30.74	184.16	34.46^{a}
	15.0	37.84	34.32	142.17	364.08	90.55^{b}
	27.0	35.60	43.78	101.49	289.97	82.29^{b}
	Amendment $\overline{X}$	31.89^{a}	24.96^{a}	76.27^{b}	268.89^{c}	
5-15 (15-25 in peat)	0.5	30.18^{a}	41.15^{ab}	47.50^{ab}	43.73^{ab}	
	15.0	39.92^{ab}	35.16^{ab}	110.16^{b}	75.73^{b}	
	27.0	34.76^{ab}	43.38^{ab}	54.38^{ab}	30.58^{a}	

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[1] Data analyzed by two-way ANOVA after ln Y transformation (MSE = .1317 and .5033 for 0-5 cm and 5-15 cm depths respectively). Time and amendment means for the 0-5 cm data, and all means for the 5-15 cm data were tested with Scheffé multiple contrasts for pairwise comparisons. Values in the time $\overline{X}$ column or amendment $\overline{X}$ row (0-5 cm data) and all values in the 5-15 cm data set, followed by the same letter do not differ significantly ($p \leq 0.05$). All means are geometric.

Table 57. The effect of depth on microbial activity (CO_2 ↑) and microbial biomass C in amended, unvegetated subalpine spoil (pathways)[1].

Parameter	Time after amendation (mo)	Amendment			
		Control	Fertilizer	Sewage sludge	Peat
Microbial activity (CO_2 ↑)	0.5	NS	**	NS	*
	15.0	NS	**	NS	*
	27.0	NS	NS	NS	*
Microbial biomass C	0.5	NS	**	**	*
	15.0	NS	NS	NS	*
	27.0	NS	NS	*	*

---

[1] See Table 42 for explanation of analysis.

NS - depths not significantly different ($p \leq 0.05$).

* - 0-5 cm depth significantly greater ($p \leq 0.05$) than 5-15 cm depth.

** - 5-15 cm depth significantly greater ($p \leq 0.05$) than 0-5 cm depth.

Table 58. White spruce root wts in amended subalpine spoil[1].

Soil depth (cm)	Time after planting (mo)	Root wt (g dwt m^{-2})			
		Control	Fertilizer	Sewage sludge	Peat
0-5	27	11.04^{a}	11.15^{a}	44.01^{ab}	112.37^{b}
5-15	27	54.60^{a}	63.33^{a}	86.17^{a}	26.13^{a}
(15-25 in peat)					

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[1] Data analyzed by one-way ANOVA (MSE = 0.78 and 1063.51 for 0-5 and 5-15 cm data respectively) and Scheffé multiple contrasts for pairwise comparisons. Values in each row, followed by the same letter(s) do not differ significantly ($p \leq 0.05$). The 0-5 cm means are geometric since the data required a ln Y transformation.

Table 59. The effect of depth on slender wheatgrass and white spruce root distribution in amended subalpine spoil 27 mo after planting[1].

	Amendment			
Plant type	Control	Fertilizer	Sewage Sludge	Peat
Slender wheatgrass	NS	NS	NS	*
White spruce	NS	NS	NS	NS

[1] Data analysis explained in Table 42.

NS - depths not significantly different ($p \leq 0.05$).

* - 0-5 cm depth root wts significantly greater ($p \leq 0.05$) than 15-25 cm root wts in the peat.

Table 60. Effect of time on % loss on ignition of amended subalpine spoil planted with white spruce[1].

Soil depth (cm)	Time after amendation (mo)	Amendment				Time $\overline{X}$
		Control	Fertilizer	Sewage Sludge	Peat	
0-5	0.5	6.02	7.07	7.59	63.62	11.97^{a}
	27.0	5.24	4.80	8.22	53.38	10.25^{a}
	Amendment $\overline{X}$	5.61^{a}	5.82^{a}	7.90^{a}	58.28^{b}	
5-15 (15-25 in peat)	0.5	7.38^{ab}	5.93^{ab}	7.40^{ab}	5.35^{ab}	
	27.0	5.09^{a}	5.80^{a}	6.76^{ab}	8.51^{b}	

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[1] Data analyzed by two-way ANOVA (MSE = .0420 and .7710 for the 0-5 and 5-15 cm depths respectively). No interaction was observed for the 0-5 cm data, hence Scheffé multiple contrasts for pairwise comparisons were applied to time and amendment means. Values in the time mean column or amendment mean row followed by the same letter do not differ significantly ($p \leq 0.05$). For the 5-15 cm data, an interaction was observed, hence Scheffé multiple contrasts for pairwise comparisons were applied to all means. Values followed by the same letter(s) do not differ significantly ($p \leq 0.05$). Geometric means are presented for the 0-5 cm data since it required a ln Y transformation.

peat amended plots was greater than that in the subsurface spoil (Table 67).

As in the unvegetated spoil, microbial activity and biomass C in the surface soil of the spruce plots were most stimulated by the addition of peat (Tables 61 and 62). Microbial activity in the 0-5 cm deep soil did not change significantly from 0.5 to 27 mo after the addition of any of the amendments (Table 61). However, the microbial biomass C in the surface soil of the peat plots increased significantly from 0.5 to 27 mo (Table 62).

In the subsurface (5-15 cm deep) soil, amendations had very little effect on either microbial activity or microbial biomass (Tables 61 and 62) at 0.5 mo following their application. Time did not significantly alter the microbial activity in any of the treatments, but did increase the level of microbial biomass C in the 5-15 cm deep sludge treated spoil. Microbial respiration and biomass C were significantly higher in the 5-15 cm deep fertilized spoil than in the 0-5 cm soil particularly at 0.5 mo after applying the fertilizer (Table 63). As expected the CO_2 efflux and microbial bimass C in the peat treated spoil were greater in the surface peat than in the spoil beneath the peat (Table 61).

3.2.3.4 Microbial development in amended spoil planted with slender wheatgrass.

The quantity of slender wheatgrass litter (27 and 39 mo after planting) sieved out of the soil samples used for microbial activity and biomass measurements (and subsequently returned) was highest in the sewage sludge treated spoil samples followed by the peat, fertilizer, and control treatments (Table 64). Shoot production by slender wheatgrass was most stimulated by the application of sewage sludge (Fig. 14), therefore resulting in greater litter input in this particular treatment. The amount of dead litter deposited on the spoil surface was not significantly different between 27 and 39 mo after planting (Table 64).

The amount of slender wheatgrass root material removed from the soil samples 27 and 39 mo after planting was significantly greater in the peat treated than in the untreated spoil (Table 65),

Table 61. Development of microbial activity ($\mu l\ CO_2 \uparrow 100g^{-1}$ dwt soil hr^{-1}) in amended subalpine spoil planted with white spruce[1].

Soil depth (cm)	Time after amendation (mo)	Amendment			
		Control	Fertilizer	Sewage Sludge	Peat
0-5	0.5	320^{ab}	190^{a}	500^{b}	1460^{c}
	27.0	210^{ab}	230^{ab}	430^{ab}	3350^{c}
5-15 (15-25 in peat)	0.5	370^{ab}	520^{ab}	500^{ab}	450^{ab}
	27.0	240^{a}	360^{ab}	580^{b}	600^{b}

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[1] Data analyzed by a two-way ANOVA (MSE = 635.19 and 818.75 for the 0-5 and 5-15 cm depths respectively). Scheffé multiple contrasts for pairwise comparisons were applied to all values in each data set. Values followed by the same letter(s) do not differ significantly ($p \leq 0.05$). The 0-5 cm data required a ln Y transformation, hence those means are geometric.

Table 62. Development of microbial biomass (mg C 100 g^{-1} dwt in soil) in amended subalpine spoil planted with white spruce[1].

Soil depth (cm)	Time after amendation (mo)	Amendment			
		Control	Fertilizer	Sewage Sludge	Peat
0-5	0.5	25.51^{a}	11.82^{a}	33.96^{ab}	185.15^{c}
	27.0	32.43^{a}	42.17^{ab}	81.52^{b}	454.29^{d}
5-15 (15-25 in peat)	0.5	30.92^{a}	41.83^{ab}	48.34^{ab}	44.54^{ab}
	27.0	34.06^{ab}	51.42^{ab}	93.27^{c}	68.71^{bc}

[1] Data analyzed by two-way ANOVA (MSE = 185.88 and 111.72 for the 0-5 and 5-15 cm depths respectively) and Scheffé multiple contrasts for pairwise comparisons. Values in each data set, followed by the same letter(s) do not differ significanty ($p \leq 0.05$).

Table 63. The effect of sampling depth on microbial activity ($CO_2 \uparrow$) and microbial biomass C in amended, subalpine spoil planted with white spruce[1].

Parameter	Time after amendation (mo)	Amendment			
		Control	Fertilizer	Sewage sludge	Peat
Microbial activity ($CO_2 \uparrow$)	0.5	NS	**	NS	*
	27.0	NS	**	NS	*
Microbial biomass C	0.5	NS	**	NS	*
	27.0	NS	NS	NS	*

[1] See Table 42 for explanation of analysis.

NS - depths not significantly different ($p \leq 0.05$).

* - 0-5 cm depth significantly greater ($p \leq 0.05$) than 5-15 cm depth.

** - 5-15 cm depth significantly greater ($p \leq 0.05$) than 0-5 cm depth.

Table 64. Slender wheatgrass litter (dead leaves and stems) input for amended subalpine spoil[1].

Time after planting (mo)	Litter input (g dwt m^{-2})				Time $\overline{X}$
	Control	Fertilizer	Sewage Sludge	Peat	
27	59.60	115.87	273.90	137.03	146.60^{a}
39	108.95	105.28	264.69	147.39	156.58^{a}
Amendment $\overline{X}$	84.27^{a}	110.57^{ab}	269.29^{c}	142.21^{b}	

---

[1] Data analyzed by two-way ANOVA (MSE = 3149.66) and Scheffé multiple contrasts for pairwise comparisons. Values in the time $\overline{X}$ column or amendment $\overline{X}$ row followed by the same letter(s) do not differ significantly ($p \leq 0.05$).

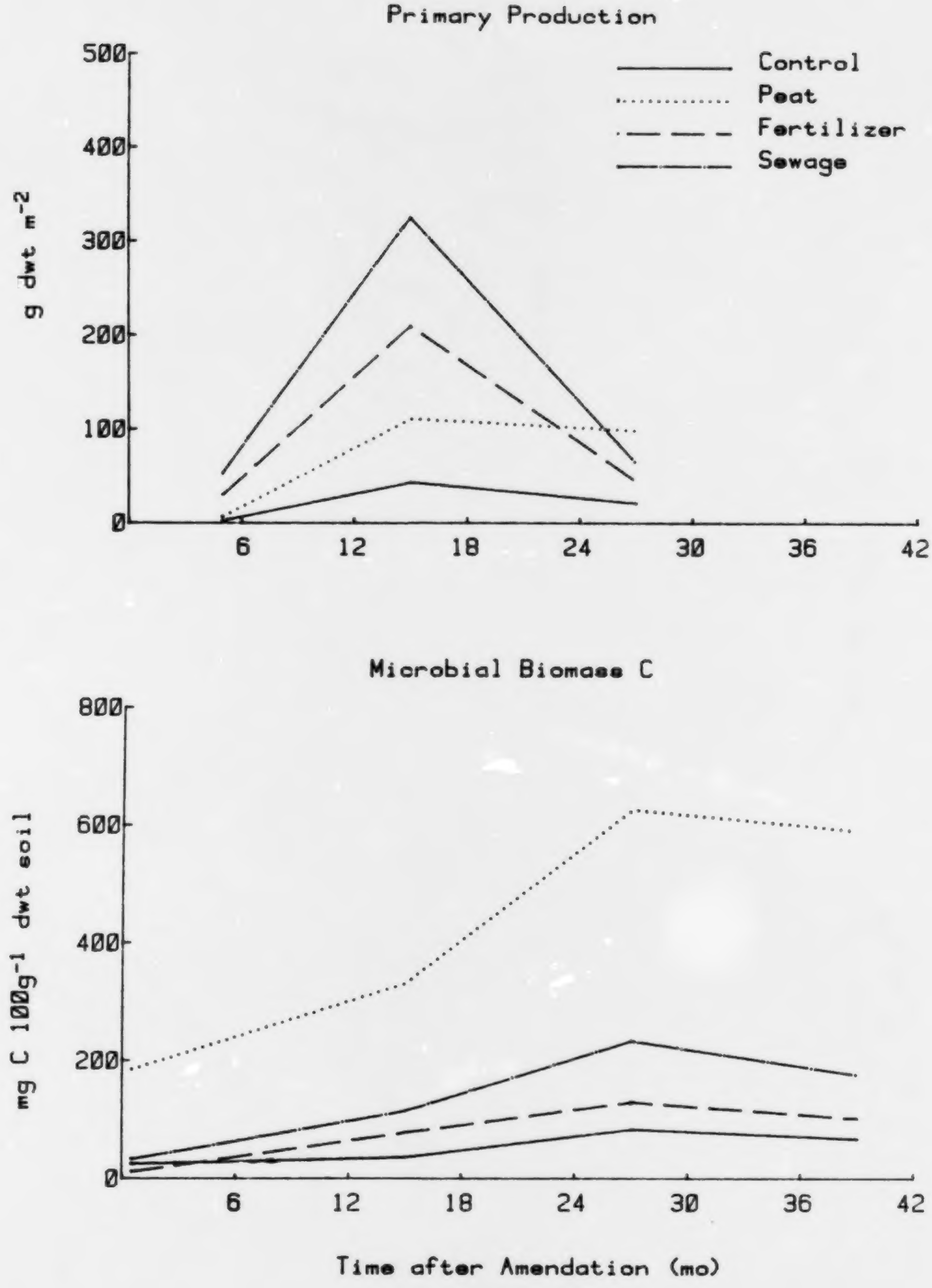

Fig. 14 Primary production and the development of microbial biomass in amended subalpine spoil planted with slender wheatgrass.

Table 65. Slender wheatgrass root wts in amended subalpine spoil[1].

Soil depth (cm)	Time after planting (mo)	Root wt (g dwt m^{-2})				Time $\overline{X}$
		Control	Fertilizer	Sewage Sludge	Peat	
0-5	27	129.75	384.93	486.00	868.56	381.05^{b}
	39	37.04	28.65	52.11	64.71	43.50^{a}
	Amendment $\overline{X}$	69.32^{a}	105.02^{ab}	159.14^{ab}	237.09^{b}	

---

[1] Data analyzed by two-way ANOVA after ln Y transformation (MSE = .5295) and Scheffé multiple contrasts for pairwise comparisons. Values in the time $\overline{X}$ column or amendment $\overline{X}$ row, followed by the same letter(s) do not differ significantly ($p \leq 0.05$). All means are geometric.

while the weight of roots in the fertilized and sewage sludge treated spoil was intermediate. The quantity of roots in all treatments was found to be significantly greater 27 mo after planting than 39 mo after planting. The root wt data should be viewed in conjunction with the shoot production estimates presented in Fig. 14. Shoot production by slender wheatgrass peaked during the second growing season and, with the exception of the peat treatment, collapsed during the third growing season. Shoot production was not measured after the fourth (39 mo) growing season. The wt of roots measured in the slender wheatgrass plots 27 mo after planting was much greater than that recorded from the white spruce plots irrespective of treatment (Tables 58 and 65). As for the white spruce roots, the wt of grass roots was not sigificantly different between the two sampling depths in any of the treatments except the peat (Table 59). Here, the quantity of roots in the surface peat was greater than that in the spoil beneath the peat.

The % loss on ignition of the 0-5 cm subalpine spoil was lowest in the untreated and fertilized spoil followed by the sewage sludge amended soil (Table 66). As in the unvegetated and spruce plot samples, peat addition resulted in the highest % loss on ignition. The % organic matter in the surface soil did not vary significantly from 0.5 to 27 mo. Loss on ignition in the subsurface soil was not strongly affected by amendation or plant growth (Table 66) over the term of the study. As expected % loss on ignition in the 0-5 cm deep soil from the peat treated plots was significantly greater than that in the spoil beneath the peat (Table 67).

Microbial activity in the surface soil from the slender wheatgrass plots was, like that from the unvegetated pathways and white spruce plots, highest in the peat treatment (Table 68). CO_2 efflux from the 0-5 cm deep peat amended spoil increased significantly from 0.5 to 15 mo after treatment. Treatment of the subalpine spoil with fertilizer and sewage sludge and planting with slender wheatgrass had no significant effect on the microbial activity - one reason being the high variability demonstrated by samples from the sewage sludge treatment in particular. Microbial respiration of the

Table 66. Effect of time on % loss on ignition of amended subalpine spoil planted with slender wheatgrass[1].

Soil depth (cm)	Time after amendation (mo)	Amendment				Time $\overline{X}$
		Control	Fertilizer	Sewage Sludge	Peat	
0-5	0.5	6.02	7.07	7.59	63.62	11.97^{a}
	15.0	7.63	6.94	8.94	67.14	13.35^{a}
	27.0	6.83	6.48	10.98	61.10	13.13^{a}
	Amendment $\overline{X}$	6.80^{a}	6.83^{a}	9.06^{b}	63.91^{c}	
5-15 (15-25 in peat)	0.5	6.38^{ab}	5.93^{a}	7.40^{ab}	5.35^{a}	
	15.0	7.83^{ab}	6.97^{ab}	7.60^{ab}	9.63^{b}	
	27.0	5.82^{a}	5.01^{a}	7.67^{ab}	9.95^{b}	

---

[1] Data analyzed by a two-way ANOVA (MSE = .0204 and .7454 for 0-5 cm and 5-15 cm respectively). Scheffé multiple contrasts for pairwise comparisons were applied to time and amendment means for the 0-5 cm data and to the individual means for the 5-15 cm data. For the 0-5 cm data, values in the time $\overline{X}$ column or amendment $\overline{X}$ row followed by the same letter do not differ significantly ($p \leq 0.05$) and for the 5-15 cm data, all values followed by the same letter(s) do not differ significantly ($p \leq 0.05$). The 0-5 cm data required a ln Y transformation, hence geometric means are presented.

Table 67. The effect of sampling depth on % loss on ignition in the amended subalpine spoil planted with slender wheatgrass and white spruce[1].

Plant type	Time after amendation (mo)	Amendment			
		Control	Fertilizer	Sewage Sludge	Peat
Slender wheatgrass	0.5	NS	NS	NS	*
	15.0	NS	NS	NS	*
	27.0	NS	NS	NS	*
White spruce	0.5	NS	NS	NS	*
	27.0	NS	NS	NS	*

[1] See Table 42 for explanation of analysis.

NS - depths not significantly different ($p \leq 0.05$).

* - 0-5 cm loss on ignition significantly greater ($p \leq 0.05$) than 5-15 cm (15-25 in the peat) loss on ignition.

Table 68. Development of microbial activity ($\mu l\ CO_2 \uparrow 100\ g^{-1}$ dwt soil hr^{-1}) in amended subalpine spoil planted with slender wheatgrass[1].

Soil depth (cm)	Time after amendation (mo)	Amendment			
		Control	Fertilizer	Sewage Sludge	Peat
0-5	0.5	320^{ab}	190^{a}	500^{ab}	1460^{b}
	15.0	290^{a}	610^{ab}	1320^{ab}	3040^{c}
	27.0	660^{ab}	930^{ab}	1440^{b}	3770^{c}
	39.0	360^{ab}	460^{ab}	800^{ab}	2790^{c}
5-15	0.5	360^{ab}	520^{ab}	490^{ab}	430^{ab}
(15-25 in peat)	15.0	340^{a}	880^{ab}	1100^{b}	830^{ab}
	27.0	400^{ab}	350^{ab}	540^{ab}	590^{ab}

[1] Data analyzed by a two-way ANOVA after ln Y transformation (MSE = 68.9 and 890.9 for the 0-5 and 5-15 cm depths respectively). A significant interaction was observed, hence Scheffé multiple contrasts for pairwise comparisons were applied to individual means. Values in each data set followed by the same letter(s) do not differ significantly ($p \leq 0.05$). Means are geometric.

surface soil from the control, fertilized and sludge treated plots did not increase significantly over the 39 mo sampling term.

Respiratory activity in the subsurface soil (5-15 cm) was significantly higher in the sewage sludge amended spoil than in the untreated spoil 15 mo after amendation (Table 68). No other treatment or time effects were detected in the deeper soil.

At 0.5 and 15 mo after amendation, CO_2 efflux in the fertilized plots was higher in the subsurface soil than in the 0-5 cm deep soil, while the 0-5 cm deep peat amended spoil was more active than the spoil beneath the peat (15-25 cm) (Table 69). Microbial respiration, 27 mo after amendation was greater in the 0-5 cm deep soil than in the 5-15 cm deep soil in all treatments.

Data on the development of microbial biomass C in the amended slender wheatgrass plots together with shoot production estimates over the first three growing seasons are presented in Table 70 and Fig. 14. The main features emerging from this data are:

i) microbial biomass C in the 0-5 cm deep soil was greatest in the peat amended spoil and remained so over the four sampling times. Over the first 27 mo of the study the microbial biomass C levels in each of the fertilizer, sewage sludge and peat treatments demonstrated an increasing trend. However, this trend did not continue into the last year of the study but instead, was maintained at the same level. Microbial biomass measurements were highest at 27 mo after amendation and planting. At this time there was a trend in biomass C levels with the largest amount occurring in the peat treatment followed by the sewage sludge, then fertilizer and untreated spoil.

ii) microbial biomass C in all treatments was highest one year after peak shoot production by slender wheatgrass (i.e. after the third growing season) (Fig. 14). Although plant production demonstrated a sharp decrease during the third growing season (27 mo), microbial biomass levels were maintained until after the fourth growing season (i.e. 39 mo after amendation).

iii) although microbial activity measurements in the deeper 5-15 cm soil did not vary over the three sampling times, microbial

Table 69. The effect of sampling depth on microbial activity (CO_2 ↑) and microbial biomass C in the amended subalpine spoil planted with slender wheatgrass[1].

Parameter	Time after amendation (mo)	Amendment			
		Control	Fertilizer	Sewage Sludge	Peat
Microbial activity (CO_2 ↑)	0.5	NS	**	NS	*
	15.0	NS	**	NS	*
	27.0	*	*	*	*
Microbial biomass C	0.5	NS	**	**	*
	15.0	NS	NS	NS	*
	27.0	*	*	*	*

---

[1] As a result of dependence between the 0-5 cm and 5-15 cm data, the effect of depth was determined by subtracting the 5-15 cm values from the 0-5 cm values and analyzing the resulting data using the model for the two-way ANOVA (i.e. microbial biomass C = $\bar{\mu}$ .. + (amendment effect) + (time effect) + error). The null hypothesis was that each treatment mean = 0 (i.e. no difference between depths). This method allows examination of each treatment in the event that not all treatments show an effect due to depth.

NS - depths not significantly different ($p \leq 0.05$).

* - 0-5 cm depth significantly greater ($p \leq 0.05$) than 5-15 cm depth.

** - 5-15 cm depth significantly greater ($p \leq 0.05$) than 0-5 cm depth.

Table 70. Development of microbial biomass (mg C 100 g^{-1} dwt soil) in amended subalpine spoil planted with slender wheatgrass[1].

Soil depth (cm)	Time after amendation (mo)	Amendment				Time $\overline{X}$
		Control	Fertilizer	Sewage Sludge	Peat	
0-5	0.5	24.83^{ab}	11.12^{a}	32.44^{ab}	184.65^{cde}	
	15.0	36.00^{a}	77.91^{abcd}	114.87^{bcde}	331.31^{f}	
	27.0	83.19^{abcde}	129.36^{bcde}	233.34^{ef}	625.85^{g}	
	39.0	66.84^{abc}	102.01^{bcde}	175.76^{def}	590.05^{g}	
5-15 (15-25 in peat)	0.5	30.18	41.15	47.50	43.73	40.08^{a}
	15.0	33.08	64.12	86.76	72.94	60.53^{b}
	27.0	53.14	61.42	121.07	97.15	78.72^{c}
	Amendment $\overline{X}$	37.57^{a}	54.52^{b}	79.31^{c}	67.67^{bc}	

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[1] Data analyzed by two-way ANOVA after $\sqrt{Y}$ (0-5 data) and ln Y (5-15 data) transformations (MSE = 2.183 and .0432 for 0-5 and 5-15 cm depths respectively). Significant differences were detected by applying Scheffé multiple contrasts for pairwise comparisons to the individual means (0-5 cm data) or to the time and amendment means (5-15 cm data). The 0-5 cm values followed by the same letter(s) do not differ significantly while the 5-15 cm values in the time $\overline{X}$ column or amendment $\overline{X}$ row followed by the same letter(s) do not differ significantly ($p \leq 0.05$). All values are geometric means.

biomass C in all treatments demonstrated a significant increase over the 27 mo term of the study (Table 70). At each of the sampling times the microbial biomass in the subsurface soil was highest in the peat and sewage sludge amended soil followed by the fertilized and then the untreated spoil.

iv) microbial biomass C measurements in both the surface and subsurface soil showed much stronger trends in microbial development than did the CO_2 efflux measurements.

The data presented in Table 69 show that 0.5 mo after treatment, biomass C in the fertilizer, sewage sludge and peat amended plots was significantly greater at 5-15 cm than at 0-5 cm; however, 27 mo after initiation of the experiment, biomass in all treatments was higher in the surface than subsurface soil.

Since % loss on ignition is a measure of organic matter and this, plus grass plant litter and roots, are sources of energy for the soil microorganisms, it was decided to test for significant relationships between these three parameters and microbial activity and biomass C measured in the slender wheatgrass plots 39 mo after planting. Product moment correlation coefficients were calculated (Table 71) and significant relationships were found between microbial activity and microbial biomass C and between % loss on ignition and microbial activity and biomass. Grass root wts correlated with litter input, but no significant correlations were observed between litter and root wts and microbial activity and biomass C. Undoubtedly better relationships would have resulted if:

i) more replicates had been tested

ii) more replicates within each amendment type had been analyzed.

3.2.3.5 Comparison of microbial development in unvegetated plots and plots planted with grass or trees. Since vegetation type, through litter production and root growth, can influence the development of organic matter, microbial activity and microbial biomass, the measurements made for these parameters in soil from the unvegetated pathways and the plots planted with white spruce and

Table 71. Product moment correlation coefficients for loss on ignition, litter input, root wt, CO_2 ↑, microbial biomass C and decomposition (% wt remaining) data collected 27 mo after amendation of the subalpine spoil (slender wheatgrass plots).

	% loss on ignition	Grass litter input	Grass root wt	Microbial activity (CO_2 ↑)	Microbial biomass C	Decomposition (% wt remaining)
% loss on ignition	1.00	.030	.206	.952*	.974*	.077
Grass litter input		1.00	.511*	.019	.110	.267
Grass root wt			1.00	.122	.238	.011
Microbial activity (CO_2 ↑)				1.00	.960*	.118
Microbial biomass C					1.00	.101
Decomposition (% wt remaining)						1.00

* - indicates significant correlation.

slender wheatgrass were compared. Data collected 27 mo after planting were used. The results obtained show that:

i) vegetation type had very little effect on the accumulation of organic matter in the various treatments. The amendments had a much greater effect (Table 72).

ii) in all but the peat treatment, microbial activity was highest in the slender wheatgrass plots. In the peat treated soil, vegetation type had no significant effect on the soil respiratory activity. The growth of white spruce in the control, fertilizer and sewage sludge treated spoil did not significantly influence CO_2 efflux (Table 73).

iii) as for microbial activity, slender wheatgrass stimulated the build-up of microbial biomass in all treatments except for the peat amended spoil. The microbial biomass in the white spruce plots, 27 mo after planting, was not significantly different from that recorded in the unvegetated plots (Table 74, Fig. 15). The results demonstrate that the development of microbial activity and biomass up until 27 mo after planting was more rapid if the fast growing slender wheatgrass, rather than the much slower growing white spruce, had been planted in the variously amended spoil.

3.2.3.6 Decomposition potential of the variously amended subalpine minespoil. Cellulose filter paper, placed in the slender wheatgrass plots and sampled after 12 and 24 mo incubation, decomposed fastest in the sludge treated plots followed by the fertilized, peat treated and control plots (Table 75). There was a significant wt loss of the filters between the 12 and 24 mo sample times.

Decay of the filters placed in the amended white spruce plots was also most rapid in the sewage sludge treatment (Table 75). However, there was no significant wt loss between 12 and 24 mo incubation in the field - presumably a result of the high degree of variability in the data set.

As has been the case for all the decomposition studies where fir wood dowel was used as a standard substrate, decay of the dowel, placed in the variously treated subalpine spoil planted with

Table 72. Effect of vegetation on % loss on ignition of the amended subalpine spoil, 27 mo after planting[1].

Plant type	Amendment				Plant type
	Control	Fertilizer	Sewage Sludge	Peat	$\overline{X}$
Unvegetated	6.38	6.16	10.06	68.51	12.83^{b}
Slender wheatgrass	6.83	6.48	10.98	61.10	13.13^{b}
White spruce	5.24	4.80	8.22	53.38	10.25^{a}
Amendment $\overline{X}$	6.11^{a}	5.77^{a}	9.69^{b}	60.68^{c}	

---

[1] Data analyzed by two way ANOVA after ln Y transformation (MSE = .0262) and Scheffé muiltiple contrasts for pairwise comparisons. Values in the plant type $\overline{X}$ column or amendment $\overline{X}$ row, followed by the same letter do not differ significantly ($p \leq 0.05$). All values are geometric means.

Table 73. Effect of vegetation on microbial activity ($\mu l\ CO_2 \uparrow 100\ g^{-1}$ dwt soil hr^{-1}) in the amended subalpine spoil, 27 mo after planting[1].

Plant type	Amendment			
	Control	Fertilizer	Sewage Sludge	Peat
Unvegetated	220^{a}	200^{a}	260^{ab}	2010^{de}
Slender wheatgrass	660^{bc}	930^{cd}	1440^{cde}	3770^{e}
White spruce	210^{a}	230^{a}	430^{a}	3350^{e}

[1] Data analyzed by two-way ANOVA after ln Y transformation (MSE = 75. 0) and Scheffé multiple contrasts for pairwise comparisons. Values are geometric means where followed by the same letter(s) do not differ significantly ($p \leq 0.05$).

Table 74. Effect of vegetation on microbial biomass C (mg C 100 g^{-1} dwt soil) in the amended subalpine spoil, 27 mo after planting[1].

Plant type	Amendment			
	Control	Fertilizer	Sewage Sludge	Peat
Unvegetated	35.60^{ab}	43.78^{abc}	101.49^{de}	289.97^{fgh}
Slender wheatgrass	83.12^{cd}	129.06^{def}	227.58^{efg}	620.40^{h}
White spruce	32.39^{a}	41.68^{abc}	81.27^{bcd}	454.60^{gh}

[1] Data analyzed by two-way ANOVA after ln Y transformation (MSE = .0475) and Scheffé multiple contrasts for pairwise comparisons. Means are geometric and where followed by the same letter(s) do not differ significantly ($p \leq 0.05$).

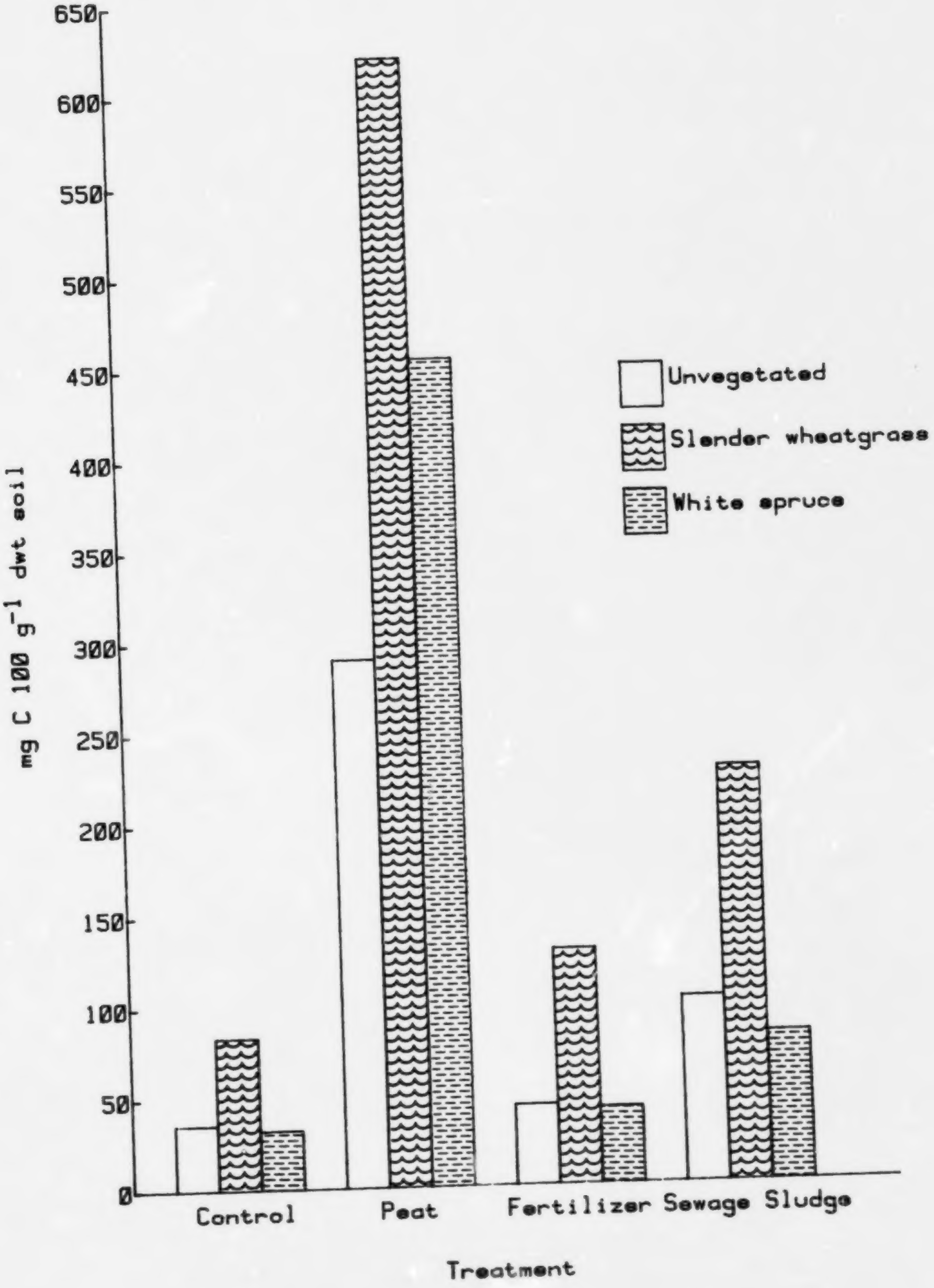

Fig. 15 Effect of vegetation on microbial biomass in the amended subalpine spoil 27 months after planting.

Table 75. The % wt remaining of cellulose filter paper placed in the amended subalpine spoil, 4 mo after planting slender wheatgrass and white spruce[1].

Plant type	Time in the field (mo)	Amendment				Time $\overline{X}$
		Control	Fertilizer	Sewage Sludge	Peat	
Slender wheatgrass	12	95	74	44	97	77.5^a
	24	76	45	4	68	48.25^b
	Amendment $\overline{X}$	85.5^c	59.5^b	24^a	83^c	
White spruce	12	99	98	46	91	83.5^a
	24	70	48	23	65	51.5^a
	Amendment $\overline{X}$	84.5^b	73^b	34.5^a	78^b	

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[1] Data analyzed by two-way ANOVA (MSE = 58.4 and 78.7 for slender wheatgrass and white spruce respectively) and Scheffé multiple contrasts for pairwise comparisons. Values in the time $\overline{X}$ columns or amendment $\overline{X}$ rows followed by the same letter do not differ significantly ($p \leq 0.05$).

slender wheatgrass or white spruce, was negligible over the term of the study (Table 76).

When the weight loss of filters placed in the slender wheatgrass plots at 4 mo after planting and then again at 28 mo after planting were compared after 12 mo incubation in the plots, it was observed that the decomposition potential of the soil for cellulose had not changed significantly since the tank study was initiated (Table 77).

The decay of slender wheatgrass leaves placed in the plots 28 mo after planting was equally fast in all treatments with 44-48% dry wt remaining after 12 mo decomposition (Table 78). The % dry wt remaining of the leaves decreased from 84 to 47% between the 6 and 12 mo sampling times.

A comparison of the % dry wt remaining of slender wheatgrass leaves and stems and cellulose filter paper after 12 mo in the field is presented in Table 79 and Fig. 16.

Application of the fertilizer, sewage sludge and peat to the subalpine spoil did not significantly influence the decomposition of slender wheatgrass leaves or stems over the short term. The leaves decayed significantly faster than the stems and both leaves and stems lost more weight than the filter paper over the 12 mo test period.

The correlation coefficients computed between % loss on ignition of the soil, grass litter input, grass root wt, microbial activity, microbial biomass C and the dry wt remaining of grass leaves after 12 mo decomposition suggested there were no significant relationships between any of the soil factors and short term decomposition (Table 71).

The effect of amendation on the decomposition of alsike clover leaves and stems placed in the clover plots and how the clover decomposition compared with the decay of slender wheatgrass litter was also studied. A three way ANOVA was applied to the data and the results are presented in Tables 80 and 81. There was no significant three-way interaction, but significant interactions were observed between litter type and plant type, and between plant type and

Table 76. The % wt remaining of fir wood dowel placed in amended subalpine spoil 4 mo after planting slender wheatgrass and white spruce[1].

Plant type	Time in the field (mo)	Amendment			
		Control	Fertilizer	Sewage Sludge	Peat
Slender wheatgrass	12	99	99	98	98
	24	98	98	97	98
White spruce	12	99	99	99	99
	24	99	99	98	98

[1] Data not analyzed due to lack of variation.

Table 77. The decomposition of cellulose filter paper (expressed as % dwt remaining) after 12 mo incubation in amended subalpine spoil planted with slender wheatgrass. Filters were placed in the plots 4 mo and 28 mo after planting[1].

Time after planting (mo)	Amendment			
	Control	Fertilizer	Sewage Sludge	Peat
4	98.8^{b}	80.1^{ab}	43.6^{a}	98.6^{b}
28	97.6^{b}	96.2^{b}	86.0^{a}	91.2^{b}

[1] Data analyzed by two-way ANOVA after $2 \times \arcsin \sqrt{p}$ transformation (MSE = .203). Significant differences were detected by Scheffé multiple contrasts for pairwise comparisons. Values are geometric and where followed by the same letter(s) do not differ significantly ($p \leq 0.05$).

Table 78. Decomposition (expressed as % dwt remaining) of slender wheatgrass leaves incubated in the amended subalpine spoil for 6 mo and 12 mo. Plots had been planted with slender wheatgrass for 28 mo prior to initiation of the experiment[1].

Incubation time	Amendment				Time
(mo)	Control	Fertilizer	Sewage Sludge	Peat	$\bar{X}$
6	87	85	82	81	84^b
12	44	47	48	47	47^a
Amendment $\bar{X}$	66^a	66^a	65^a	64^a	

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[1] Data analyzed by two-way ANOVA (MSE = 15.6) and Scheffé multiple contrasts for pairwise comparisons. Values in the time $\bar{X}$ column or amendment $\bar{X}$ row, followed by the same letter, do not differ significantly ($p \leq 0.05$).

Table 79. The decomposition (expressed as % dwt remaining after 12 mo in the field) of slender wheatgrass stems and leaves and cellulose filter paper placed in amended subalpine spoil 28 mo after planting slender wheatgrass[1].

Litter type	Amendment				Litter type $\overline{X}$
	Control	Fertilizer	Sewage Sludge	Peat	
Leaves	44	47	48	47	47^a
Stems	77	78	73	78	77^b
Filter paper	97	92	84	87	90^c
Amendment $\overline{X}$	73^a	72^a	68^a	71^a	

---

[1] Data analyzed by two-way ANOVA (MSE = 51.3) and Scheffé multiple contrasts for pairwise comparisons. Values in the litter type $\overline{X}$ column or amendment $\overline{X}$ row, followed by the same letter do not differ significantly ($p \leq 0.05$).

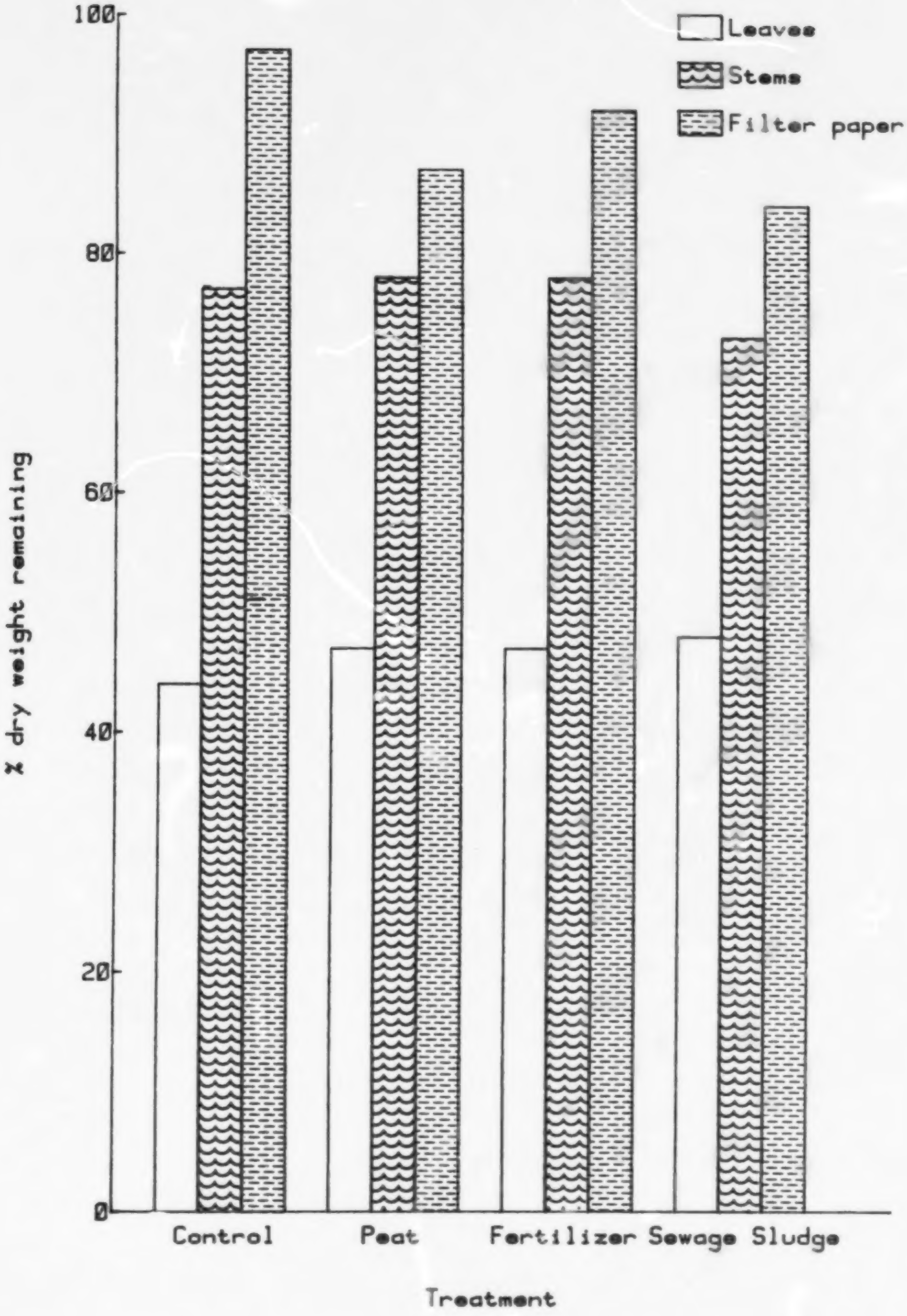

Fig. 16 The decomposition of slender wheatgrass leaves and stems and cellulose filter paper placed in amended subalpine spoil 28 months after planting slender wheatgrass.

Table 80. Three-way ANOVA table to test the effect of litter type (i.e. stems or leaves), plant type (i.e. slender wheatgrass or alsike clover) and amendment (i.e. control, fertilizer, sewage sludge and peat) on % dry litter remaining after 12 mo incubation in the subalpine spoil.

Source	SS	df	MS	F	prob.[1]
Litter type	13680.4	1	13680.4	915.84	***
Plant type	11353.5	1	11353.5	760.06	***
Amendment	208.9	3	69.6	4.66	**
Litter type x plant type	900.4	1	900.4	60.28	***
Litter type x amendment	114.5	3	38.2	2.56	NS
Plant type x amendment	187.8	3	62.6	4.19	**
Litter type x plant type x amendment	28.9	3	9.6	.64	NS
Error	1195.0	80	14.9		
Total	27669.4	95			

[1] * - significant at $p \leq 0.05$.

** - significant at $p \leq 0.01$.

*** - significant at $p \leq 0.001$.

NS - not significant.

Table 81. The effect of amendation on the decomposition (expressed as % dry litter remaining after 12 mo incubation) of slender wheatgrass and alsike clover litter placed in the amended subalpine spoil, 28 mo after planting (extension of data presented in Table 80)[1].

Treatment	Plant or litter type	Amendment			
		Control	Fertilizer	Sewage Sludge	Peat
% dry wt stems or leaves remaining	Clover leaves	29	28	33	33
	Clover stems	47	45	48	54
% dry wt stems or leaves remaining	Grass leaves	44	47	47	47
	Grass stems	77	78	73	78
% dry stem and leaf wt remaining (plant type x amendment means)	Clover (leaves plus stems)	38^{ab}	36^{a}	40^{ab}	43^{b}
	Grass (leaves plus stems)	60^{c}	62^{c}	60^{c}	62^{c}
% dry clover and grass leaf or stem wt remaining (litter type x amendment means)[1]	Leaves (clover plus grass)	37^{a}	37^{a}	40^{a}	40^{a}
	Stems (clover plus grass)	62^{b}	61^{b}	60^{b}	66^{b}

(cont'd)

Table 81. (cont'd).

Treatment	Plant or litter type	% dry wt remaining (summed)	
		Leaves	Stems
% dry leaf or stem wt remaining when summed over amendments (litter type x plant type means)[1]	Clover	31^a	48^b
	Slender wheatgrass	46^b	76^c

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[1] Means tested by Scheffé multiple contrasts for pairwise comparisons. Values in each treatment followed by the same letter(s) do not differ significantly ($p \leq 0.05$).

amendment (Table 78). Therefore to locate significant differences, Scheffé multiple contrasts for pairwise comparisons were applied to the individual means obtained for each amendment type where the treatments interacted. Where there was no interaction, Scheffé multiple contrasts were applied to the factor means. The results presented in Table 81 show that:

i) clover leaves and stems combined, decayed more rapidly than slender wheatgrass leaves and stems (see % dry stem and leaf wt remaining treatment). Clover litter lost more wt in the fertilized clover plots than in the peat treated plots, whereas amendation had no significant effect on the wt loss of slender wheatgrass litter.

ii) the leaves of clover and grass combined decomposed quicker than the stems (see % dry clover and grass leaf or stem wt remaining treatment). Amendation did not significantly influence the decay of the leaves or the stems.

iii) the % dry leaf or stem wt remaining of either clover or slender wheatgrass when summed over all the amendments demonstrated that leaves of either clover or grass decomposed faster than their respective stems. However clover stems and slender wheatgrass leaves did not differ in their wt loss after 12 mo incubation. Clover leaves decayed most rapidly of all the litter types tested (i.e. grass leaves and stems and clover stems).

3.2.3.7 N_2 fixation by alsike clover. The N_2 (C_2H_2) fixation capacity of alsike clover grown in the variously amended subalpine spoil was tested at the conclusion of the first three growing seasons. As was the case for the rambler alfalfa in the grasslands spoil, the results were highly variable resulting in no significant amendment effects on N_2 fixation (Table 82). Although not tested, N_2 fixation appeared to be greater after the second growing season than at the other two sample times.

Partial correlation coefficients were calculated to determine if any strong relationships existed amongst shoot wt, root wt, shoot N, total soil N, soil NO_3-N and N_2 fixation capacity

Table 82. N_2 (C_2H_2) fixation capacity (nmoles g^{-1} dry root hr^{-1}) of alsike clover in subalpine spoil tank plots[1].

Treatment	Time after planting		
	3 mo	14 mo	26 mo
Control	288.2^{a}	571.4^{a}	274.1^{a}
Fertilizer	399.9^{a}	503.6^{a}	121.5^{a}
Sewage Sludge	66.0^{a}	395.2^{a}	75.0^{a}
Peat	0^{a}	625.1^{a}	44.9^{a}
MSE	73976.0	NA	20551

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[1] A one-way ANOVA was applied to the 3 mo and 26 mo data. Peat values in the 3 mo data set were excluded from the analysis because they had 0 variance. The hypothesis that the means of the amendments were not different from the non-random mean, 0, was tested using the confidence interval ($\pm$ 526.8). Values in each column, followed by the same letter do not differ significantly ($p \leq 0.05$).

[2] Data analyzed with a Kruskal-Wallis non-parametric test.

after the second growing season. Except for root and shoot wt, there were no strong relationships amongst any of the parameters (Table 83). Again more replication particularly within each amendment type and the determination of N levels in soil around the root systems for which N_2 fixation was tested would have resulted in better correlations.

3.2.4. Oil Sand Tailings - Effects of Amendation and Plant Growth on Microbial Development.

3.2.4.1 Microbial characteristics 0.5 mo and 15 mo after amendation and planting. Immediately after amendation, the peat treated sand appeared to have the highest level of ATP although results were extremely variable (Table 84). In the untreated and fertilized sand, ATP occurred in such low quantities that it could not be measured.

Although the presence of microbes in the control and fertilized sand was not detected through ATP measurements, the dilution plating technique and direct observation demonstrated that the presence of bacteria, actinomycetes and fungal hyphae in these soils (Tables 85, 86, 87 and 88). Sewage sludge and peat amendation of the sand significantly increased the number of bacteria in both the 0-5 and 5-15 cm deep soil, while fertilization had no effect (Table 85). Fifteen months after amendation and planting (slender wheatgrass), bacterial counts from the 0-5 cm peat and sewage sludge treated soil had not changed significantly and were still greater than counts from the untreated sand. However, in the subsurface soil, bacteria in the peat treatment decreased significantly from 0.5 to 15 mo after application, while those in the other three treatments appeared to be unaffected by time.

The application of peat also introduced a significant number of actinomycetes, while sewage sludge and fertilizer had a negligible effect on the presence of actinomycetes in the tailing sand (Table 86). At the conclusion of the second growing season (15 mo) actinomycete counts in the 5-15 cm sewage sludge amended sand had

Table 83. Partial correlation coefficients for determining the relationships amongst shoot wt, root wt, shoot N, total soil N, soil NO_3-N and N_2 (C_2H_2) fixation capacity for alsike clover after 1978 growing season.

	Shoot wt (g)	Root wt (g)	Shoot N (%)	Total Soil N (%)	Soil NO_3-N (µg/g dry soil)	N_2 (C_2H_2) fixation (nmoles/g root/hr)
Shoot wt (g)		.85	-.57	-.20	-.36	-.13
Root wt (g)			-.50	-.27	-.48	-.30
Shoot N (%)				.17	.48	.16
Total Soil N (%)					.65	-.51
Soil NO_3-N (µg/g dry soil)						-.10
N_2 (C_2H_2) fixation (nmoles/g dry root/hr)						

Table 84. ATP levels in extracted oil sand 0.5 mo after amendation. Values are $\overline{X} \pm$ SD.

Amendment	Soil depth (cm)	ATP (μg 100g^{-1} dry soil)
Control	0-5	0
	5-15	0
Fertilizer	0-5	0
	5-15	0
Sewage Sludge	0-5	2.30 ± 3.56
	5-15	2.40 ± 4.16
Peat	0-5	27.0 ± 14.12
	15-25	0.17 ± 0.15

Table 85. Effect of amendation and time on bacterial numbers in extracted oil sands tank plots[1]. Plots were planted with slender wheatgrass.

Amendment	Depth (cm)	No. of bacteria x 10^6 g^{-1} dwt soil	
		Time after amendation (mo)	
		0.5	15
Control	0-5	7.48^{a}	8.43^{ab}
Fertilizer		7.86^{ab}	68.57^{bc}
Sewage Sludge		1204.31^{d}	220.27^{cd}
Peat		649.97^{d}	600.63^{cd}
Control	5-15	8.68^{a}	9.43^{a}
Fertilizer		8.78^{a}	12.59^{ab}
Sewage Sludge		110.07^{c}	127.70^{c}
Peat	15-25	72.29^{bc}	10.89^{a}

[1] Data analyzed by a two-way ANOVA (MSE = .389 and .281 for 0-5 cm and 5-15 cm respectively) after a ln Y transformation. A significant interaction was detected for both data sets, hence Scheffé multiple contrasts for pairwise comparisons were applied to all values. All values are geometric and those followed by the same letter(s) do not differ significantly ($p \leq 0.05$).

Table 86. Effect of amendation and time on actinomycete numbers in extracted oil sands tank plots[1]. Plots were planted with slender wheatgrass.

Amendment	Depth (cm)	No. of actinomycetes x 10^4 g^{-1} dwt soil	
		Time after amendation (mo)	
		0.5	15
Control	0-5	0.62^a	4.21^{ab}
Fertilizer		0.96^a	0.97^a
Sewage Sludge		2.18^{ab}	20.44^b
Peat		3281.75^c	1220.40^c
Control	5-15	0^a	0.90^{ab}
Fertilizer		0.28^a	0.34^a
Sewage Sludge		0.63^a	18.31^b
Peat	15-25	332.99^d	33.91^{cd}

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[1] Data analyzed by a two-way ANOVA (MSE = .369 and .473 for 0-5 cm and 5-15 cm data respectively) after a ln Y transformation. A significant interaction was detected for both data sets, hence Scheffé multiple contrasts for pairwise comparisons were applied to all values. All values are geometric and those followed by the same letter(s) do not differ significantly ($p \leq 0.05$).

Table 87. Hyphal lengths in 0-5 cm oil sands tailings before planting (0.5 mo) and after planting slender wheatgrass (15 mo)[1].

Hyphal category ($m\ g^{-1}$ dwt soil)	Sampling time (mo)	Amendment			
		Control	Fertilizer	Sewage Sludge	Peat
Total	0.5	95^{a}	37^{a}	86^{a}	1304^{b}
	15.0	41^{a}	57^{a}	66^{a}	675^{b}
Total with cell contents	0.5	77^{ab}	15^{a}	59^{a}	248^{c}
	15.0	27^{a}	36^{a}	24^{a}	158^{bc}

[1] Data in each hyphal category were analyzed by a two-way ANOVA (MSE = .087 and 769.98 for total and total with cell contents respectively) and Scheffé multiple contrasts for pairwise comparisons. Values followed by the same letter(s) do not differ significantly ($p \leq 0.05$).

Table 88. Hyphal lengths in 5-15 cm (15-25 cm in peat) oil sands tailings (0.5 mo) and after planting slender wheatgrass (15 mo)[1].

Hyphal category ($m\ g^{-1}$ dwt soil)	Sampling time (mo)	Amendment				Time
		Control	Fertilizer	Sewage Sludge	Peat	$\overline{X}$
Total	0.5	103^{ab}	46^{a}	89^{ab}	356^{b}	
	15.0	39^{a}	26^{a}	53^{a}	34^{a}	
Total with cell contents	0.5	81	9	60	81	43
	15.0	26	20	28	15	22
Amendment $\overline{X}$		46	13	41	35	

[1] Data in each hyphal category were analyzed by a two-way ANOVA (MSE = .245 and 62.02 for total and total with cell contents respectively). The total with cell contents data required a ln Y transformation. Scheffé multiple contrasts for pairwise comparisons were applied to all values in the total category (a significant interaction was detected) and to the time $\overline{X}$ and amendment $\overline{X}$ values in the total with cell contents category.

Values in the total category followed by the same letter(s) do not differ significantly ($p \leq 0.05$). Values in the time $\overline{X}$ column or amendment $\overline{X}$ row in the total with cell contents category, followed by the same letter do not differ significantly ($p \leq 0.05$).

increased significantly compared with the 0.5 mo measurements but those in the other treatments had not changed.

Hyphal length measurements (both total and that with cell contents) were, like the bacteria and actinomycete numbers, highest in the 0-5 cm peat treated tailing sand at both sampling times (Table 87). No differences in mycelial lengths were observed between the surface sand in the control plots and that in the fertilized and sewage sludge treatments. In the deeper, subsurface soil, peat amendation did not have as significant an effect on mycelial lengths as was observed in the 0-5 cm soil (Table 88). By 15 mo after amendation and planting, total mycelial lengths in the 5-15 cm deep sand did not vary amongst the four treatments. However, hyphae having cell contents decreased significantly over the 15 mo study period (Table 88).

3.2.4.2 Microbial development in amended, unvegetated oil sand tailings. The application of peat and sewage sludge to the oil sands spoil significantly raised the organic matter level of the spoil (Table 89). The organic matter content in the four treatments did not vary 0.5 to 39.0 mo after amendation.

The microbial activity and biomass C in the unvegetated sand followed the same pattern as that observed for the % loss on ignition measurements, i.e. the peat treatment was the most active and contained the largest quantity of microbial biomass followed by the sewage sludge treated sand (Tables 90 and 91). Fertilization did not significantly affect microbial activity and biomass C. In general, CO_2 efflux and microbial biomass were highest at the 15 mo sampling time, but then decreased to levels not significant from the 0.5 mo measurements (Tables 90 and 91).

3.2.4.3 Microbial development in amended tailings sand planted with jack pine. Application of sewage sludge to the extracted oil sand stimulated the greatest amount of root production by jack pine in comparison with root wts produced in the other treatments (Table 92). This was true at both sampling depths. Root wts increased

Table 89. Effect of time on % loss on ignition of amended, unvegetated oil sands tailings (pathways)[1].

Soil depth (cm)	Time after amendation (mo)	Amendment				Time $\overline{X}$
		Control	Fertilizer	Sewage sludge	Peat	
0-5	0.5	.40	.27	1.40	26.83	1.43^a
	27.0	.23	.27	1.46	27.56	1.26^a
	39.0	.26	.20	2.10	24.41	1.27^a
	Amendment $\overline{X}$	$.29^a$	$.24^a$	1.65^b	26.24^c	

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[1] Data analyzed by two-way ANOVA after ln Y transformation (MSE = 0.24). No interaction was determined, hence Scheffé multiple contrasts for pairwise comparisons were applied to amendment and time means. Values in the time $\overline{X}$ column or amendment $\overline{X}$ row, followed by the same letter, do not differ significantly ($p \leq 0.05$). All means are geometric.

Table 90. Development of microbial activity ($\mu l\ CO_2 + 100\ g^{-1}$ dwt hr^{-1}) in variously amended oil sands tailings (pathways)[1].

Soil depth (cm)	Time after amendation (mo)	Amendment				Time $\overline{X}$
		Control	Fertilizer	Sewage Sludge	Peat	
0-5	0.5	70	40	200	940	140^a
	15.0	90	90	240	1850	250^b
	27.0	50	60	90	1050	130^a
	39.0	60	70	180	1010	160^a
	Amendment $\overline{X}$	60^a	60^a	170^b	1170^c	

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[1] Data analyzed by two-way ANOVA after ln Y transformation (MSE = 150.0). No interaction was observed, hence Scheffé multiple contrasts for pairwise comparisons were applied to the amendment and time means. Values in the amendment $\overline{X}$ row or time $\overline{X}$ column, followed by the same letter(s) do not differ significantly ($p \leq 0.05$). All values are geometric.

Table 91. Development of microbial biomass (mg C 100 g^{-1} dwt soil) in variously amended oil sands tailings (pathways)[1].

Soil depth (cm)	Time after amendation (mo)	Amendment				Time $\bar{X}$
		Control	Fertilizer	Sewage Sludge	Peat	
0-5	0.5	3.85	1.57	17.22	90.26	9.84^a
	15.0	6.24	5.86	16.06	158.61	17.47^b
	27.0	4.13	4.13	14.04	151.26	13.79^{ab}
	39.0	3.97	3.62	23.60	121.56	14.25^{ab}
	Amendment $\bar{X}$	4.45^a	3.42^a	17.40^b	127.38^c	

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[1] Data analyzed by a two-way ANOVA after ln Y transformation (MSE = 0.22). No interaction was observed, hence Scheffé multiple contrasts for pairwise comparisons were applied to the amendment and time means. Values in the amendment $\bar{X}$ row or time $\bar{X}$ column, followed by the same letter(s) do not differ significantly ($p \leq 0.05$). All values are geometric.

Table 92. Jack pine root wts in amended oil sands tailings[1].

Soil depth (cm)	Time after planting (mo)	Root wt (g dwt m^{-2})				Time $\overline{X}$
		Control	Fertilizer	Sewage Sludge	Peat	
0-5	27.0	0	.51	4.42	.58	0.89^a
	39.0	1.05	2.36	39.63	11.26	6.65^b
Amendment $\overline{X}$		0.43^a	1.25^{ab}	13.84^c	3.41^b	
5-15	27.0	0	1.93	14.49	0.61	1.92^a
(15-25 in peat)	39.0	2.34	20.12	148.23	6.59	15.81^b
Amendment $\overline{X}$		0.83^a	6.87^b	47.08^c	2.49^{ab}	

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[1] Data analyzed by two-way MANOVA after ln (Y + 1) transformation (MSE = 11.93 and 15.52 for 0-5 cm and 5-15 cm depths respectively). In each data set values in the time $\overline{X}$ column or amendment $\overline{X}$ row followed by the same letter(s) do not differ significantly ($p \leq 0.05$) as determined by Scheffé multiple contrasts for pairwise comparisons. All means are geometric.

significantly between 27.0 and 39.0 mo after planting in all treatments (Table 92). At 27 mo after planting, jack pine root wts did not differ between the 0-5 m and 5-15 cm deep soil but by 39 mo after planting, roots in the surface fertilized and sewage sludge treated sand were heavier than those in the subsurface soil (Table 93).

Microbial respiration and biomass C levels measured in the amended jack pine plots on four different occasions after amendation and planting are presented in Tables 94 to 97. The main features emerging from this data are:

i) with the exception of the peat amended sand, where CO_2 efflux over the term of the study was always greater than the fertilized and untreated sand, there were no obvious differences in microbial respiration in the surface soil of the various treatments (Table 94). Once the amendments had been applied, microbial activity did not change significantly over the four sampling times suggesting that the growth of jack pine did not influence the development of microbial activity in the 0-5 cm soil.

ii) in the subsurface soil CO_2 efflux 15 mo after amendation was significantly greater in the peat treated sand than in the fertilized, sewage sludge treated and untreated sand (Table 94). No amendation effects were observed at any of the other sample times. As in the surface soil, microbial activity did not change significantly over the 39 mo study period.

iii) in the 0-5 cm deep soil, microbial biomass C levels, in contrast to CO_2 efflux, demonstrated more obvious effects of amendation (Table 96). Immediately after application of the amendments (i.e. 0.5 mo) biomass in the peat treatment was significantly higher than in the sewage sludge treated sand followed by the fertilized and untreated sand. This was also the case 39 mo after amendation and planting. Like the respiration results, biomass C did not demonstrate significant increases or decreases over the 39 mo study.

Table 93. The effect of depth on jack pine root distribution in amended oil sand tailings[1].

Time after planting (mo)	Amendment			
	Control	Fertilizer	Sewage Sludge	Peat
27.0	NS	NS	NS	NS
39.0	NS	*	*	NS

---

[1] See Table 42 for explanation of statistical analysis.

NS - depths not significantly different ($p \leq 0.05$).

* - 0-5 cm root wt significantly greater ($p \leq 0.05$) than 5-15 cm (15-25 in peat) root wt.

Table 94. Development of microbial activity ($\mu l\ CO_2 + 100\ g^{-1}\ dwt\ hr^{-1}$) in variously amended oil sands tailings planted with jack pine[1].

Soil depth (cm)	Time after amendation (mo)	Amendment			
		Control	Fertilizer	Sewage Sludge	Peat
0-5	0.5	70^{ab}	40^{a}	200^{abc}	940^{cde}
	15.0	50^{ab}	80^{ab}	240^{abcd}	1740^{de}
	27.0	50^{ab}	60^{ab}	430^{bcde}	2150^{e}
	39.0	50^{ab}	80^{ab}	250^{abcd}	1340^{de}
5-15	0.5	60^{abc}	60^{abc}	60^{abc}	320^{cd}
	15.0	50^{ab}	70^{abc}	140^{bc}	440^{d}
	27.0	40^{ab}	50^{ab}	110^{bcd}	100^{bcd}
	39.0	20^{a}	40^{ab}	70^{ab}	40^{ab}

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[1] Data analyzed in the same way as that described in Table 68. MSE = 9490 and 8060 for 0-5 and 5-15 cm depths respectively. Values for each depth followed by the same letter(s) do not differ significantly ($p \leq 0.05$). Data required a ln Y transformation, hence all means are geometric.

Table 95. The effect of sampling depth on microbial activity (CO_2 ↑) in the amended oil sands tailings planted with jack pine[1].

Time after amendation (mo)	Amendment			
	Control	Fertilizer	Sewage Sludge	Peat
0.5	NS	NS	*	*
15.0	NS	NS	NS	*
27.0	NS	NS	*	*
39.0	*	*	*	*

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[1] See Table 42 for explanation of statistical analysis.

NS - depths not significantly different ($p \leq 0.05$).

* - 0-5 cm CO_2↑ significantly greater ($p \leq 0.05$) than 5-15 cm (15-25 in the peat) CO_2 ↑.

Table 96. Development of microbial biomass (mg C 100 g^{-1} dwt soil) in variously amended oil sands tailings planted with jack pine[1].

Soil depth (cm)	Time after amendation (mo)	Amendment			
		Control	Fertilizer	Sewage Sludge	Peat
0-5	0.5	3.85^{ab}	1.57^{a}	17.22^{cd}	90.26^{ef}
	15.0	3.24^{ab}	7.18^{bc}	20.31^{cde}	181.29^{f}
	27.0	3.45^{ab}	7.36^{bc}	27.82^{cde}	197.93^{f}
	39.0	8.57^{bc}	9.80^{bc}	39.35^{de}	215.53^{f}
5-15 (15-25 in peat)	0.5	4.73^{abc}	2.77^{ab}	2.77^{ab}	22.67^{c}
	15.0	3.14^{ab}	5.28^{abc}	7.17^{abc}	28.87^{c}
	27.0	2.86^{ab}	4.11^{abc}	12.03^{bc}	6.26^{abc}
	39.0	2.06^{a}	3.04^{ab}	10.62^{bc}	4.58^{abc}

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[1] Data analyzed in the same way as that described in Table 68. MSE = 5.77 and 9.02 for 0-5 cm and 5-15 cm depths respectively. Values for each depth followed by the same letter(s) do not differ significantly ($p \leq 0.05$). Data required a ln Y transformation, hence all means are geometric.

Table 97. The effect of sampling depth on microbial biomass C in the variously amended oil sands tailings planted with jack pine.

Time after amendation (mo)	Amendment			
	Control	Fertilizer	Sewage Sludge	Peat
0.5	NS	NS	*	*
15.0	NS	NS	*	*
27.0	NS	*	*	*
39.0	*	*	*	*

[1] See Table 42 for explanation of analysis.

NS - biomass C in the two depths not significantly different ($p \leq 0.05$).

* - biomass C in the 0-5 cm soil significantly greater ($p \leq 0.05$) than that in 5-15 cm (15-25 in the peat) soil.

iv) microbial biomass C in the subsurface soil of the jack pine plots was not significantly influenced by amendation or time (Table 96).

v) both microbial activity and biomass in the sludge and peat treated plots tended to be higher in the surface than subsurface soil except at 39 mo when all treatments demonstrated more CO_2 efflux and biomass in the surface soil (Tables 95 and 97).

3.2.4.4 Microbial development in amended tailings sand planted with slender wheatgrass. Litter input by slender wheatgrass grown in the fertilized, sewage sludge and peat treated sand was not significantly different amongst the three amendments with no differences recorded between the 27 and 39 mo inputs (Table 98). Data were highly variable.

Roots, however, tended to be heavier in the 0-5 cm deep peat and sludge treated soil than in the fertilized and control soil, particularly at 39 mo after planting (Table 99). In the subsurface, soil root wts in the fertilizer and sewage sludge treated plots were greater than in the untreated sand.

Twenty seven months after planting, there were more grass roots in the 0-5 cm deep fertilized and peat amended sand than in the deeper soil while sewage sludge and peat treated plots had more roots in the surface than subsurface soil at 39 mo after planting (Table 100).

Loss on ignition measured in the 0-5 cm surface soil from the slender wheatgrass plots followed a similar pattern to that obtained for the 0-5 cm unvegetated sand. Thirty nine months after the initiation of the study organic matter levels were highest in the peat and sewage sludge treated plots (Table 101). The % loss on ignition in the surface soil at 39 mo was not significantly altered from that at 0.5 mo after amendation. In the deeper soil, amendation had no well defined effects on the organic matter estimates made at any of the sampling times (Table 101). Shortly after amendation, organic matter in only the peat treated plots was greater in the surface than surface soil; however, at the conclusion of the study %

Table 98. Slender wheatgrass litter (dead leaves and stems) input for variously amended oil sands tailings[1].

Time after planting (mo)	Litter input (g dwt m^{-2})				Time $\overline{X}$
	Control	Fertilizer	Sewage Sludge	Peat	
27	.69	126.89	113.77	80.74	36.75^a
39	.23	59.70	396.87	175.83	46.86^a
Amendment $\overline{X}$	$.44^a$	87.11^b	212.69^b	119.23^b	

[1] Data analyzed by two-way ANOVA after ln (Y + 1) transformation (MSE = 0.71). No interaction was observed, hence Scheffé multiple contrasts for pairwise comparisons were applied to the amendment and time means. Values in the time $\overline{X}$ column or amendment $\overline{X}$ row, followed by the same letter, do not differ significantly ($p \leq 0.05$). All means are geometric.

Table 99. Slender wheatgrass root wts in amended oil sands tailings[1].

Soil depth (cm)	Time after planting (mo)	Root wt (g dwt m^{-2})			
		Control	Fertilizer	Sewage Sludge	Peat
0-5	27	3.06^{ab}	63.43^{c}	43.82^{cd}	44.70^{cd}
	39	1.23^{a}	18.17^{bc}	126.47^{d}	94.63^{d}
5-15 (15-25 in peat)	27	1.90^{a}	25.03^{cd}	38.86^{cd}	5.10^{abc}
	39	1.82^{a}	12.55^{bcd}	51.42^{d}	3.22^{ab}

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[1] Data was analyzed by a two-way MANOVA after ln Y transformation (MSE = 19.78 and 15.20 for 0-5 and 5-15 cm depths respectively). Values in each data set followed by the same letter(s) do not differ significantly ($p \leq 0.05$) as determined by Scheffé multiple contrasts for pairwise comparisons. All values are geometric.

Table 100. The effect of depth on slender wheatgrass root distribution in amended oil sands tailings[1].

Time after planting (mo)	Amendment			
	Control	Fertilizer	Sewage Sludge	Peat
27.0	NS	*	NS	*
39.0	NS	NS	*	*

[1] See Table 42 for explanation of statistical analysis.

NS - depths not significantly different ($p \leq 0.05$).

* - 0-5 cm root wt significantly greater ($p \leq 0.05$) than 5-15 cm (15-25 in peat) root wt.

Table 101. Effect of time on % loss on ignition of amended oil sands tailings planted with slender wheatgrass[1].

Soil depth (cm)	Time after amendation (mo)	Amendment			
		Control	Fertilizer	Sewage Sludge	Peat
0-5	0.5	$.40^{ab}$	$.27^{a}$	1.46^{bcd}	26.83^{e}
	15.0	$.41^{ab}$	$.29^{a}$	$.69^{abc}$	66.02^{e}
	27.0	$.27^{a}$	$.41^{ab}$	2.62^{cd}	40.04^{e}
	39.0	$.38^{a}$	$.54^{ab}$	2.54^{d}	41.68^{e}
5-15 (15-25 in peat)	0.5	$.31^{abc}$	$.29^{ab}$	$.46^{abc}$	3.06^{d}
	15.0	$.33^{abc}$	$.17^{a}$	$.47^{abc}$	$.51^{abc}$
	27.0	$.35^{abc}$	$.33^{abc}$	$.82^{bcd}$	1.23^{cd}
	39.0	$.27^{a}$	$.30^{ab}$	$.56^{abc}$	$.44^{abc}$

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[1] Data analyzed by a two-way MANOVA after ln Y transformation (MSE = 4.94 and 5.04 for 0-5 and 5-15 cm data respectively). Significant differences amongst the values in each data set were determined by Scheffé multiple contrasts for pairwise comparisons. Values followed by the same letter(s) do not differ significantly ($p \leq 0.05$). All means are geometric.

loss on ignition in all treatments except the control plots was higher in the 0-5 cm than 5-15 cm deep soil (Table 102).

The microbial activity in the surface soil of the amended slender wheatgrass plots followed the same trends as observed in the jack pine plots. Immediately after amendation, CO_2 efflux from the untreated and fertilized sand tended to be lower than that from the sewage sludge and peat amended plots (Table 103). Although microbial activity in the fertilized and peat treated sand appeared to increase over the first 15 mo of the study, this increase was not significant - a result of extreme variation. Time did not have a significant effect on the microbial activity so that at the end of the study period CO_2 evolution from the peat treatment was still greater than that from the sludge and fertilizer treatments (Table 103).

As in the jack pine plots, amendation had no obvious effects on microbial respiration in the subsurface soil (Table 103). CO_2 efflux from the 0-5 cm sewage sludge and peat amended sand was greater than that from the subsurface soil regardless of sampling time. At 39 mo after amendation and planting, microbial activity in the 0-5 cm deep soil from the untreated and fertilized plots was also greater than that in the subsurface soil (Table 104). Similar data were obtained for the jack pine planted soil.

In contrast to the CO_2 efflux measured for the 0-5 cm deep soil, microbial biomass C estimates for the same soils demonstrated an increasing trend over the first 27 mo of the study (Table 105, Fig. 17). This trend did not apply to the untreated sand. Also no trends in biomass development were obtained for soil from the jack pine plots suggesting that the increasing trend in microbial biomass C in the amended slender wheatgrass plots was related to the productivity of the grass (particularly in the sewage sludge and peat treatments - see Fig. 17 for slender wheatgrass primary production data). Microbial activity measurements demonstrated a lack of well-defined differences amongst the various treatments; conversely the microbial biomass C estimates obtained for each of the treatments at 39 mo after planting were all significantly

Table 102. The effect of sampling depth on % loss on ignition in the amended oil sands tailings planted with slender wheatgrass[1].

Time after amendation (mo)	Amendment			
	Control	Fertilizer	Sewage Sludge	Peat
0.5	NS	NS	NS	*
15.0	NS	NS	NS	*
27.0	NS	NS	*	*
39.0	NS	*	*	*

---

[1] See Table 42 for explanation of statistical analysis.

NS - depths not significantly different ($p \leq 0.05$).

* - 0-5 cm loss on ignition significantly greater ($p \leq 0.05$) than 5-15 cm loss on ignition.

Table 103. Development of microbial activity (μ l CO_2 + 100 g^{-1} dwt soil hr^{-1}) in variously amended oil sands tailings planted with slender wheatgrass[1].

Soil depth (cm)	Time after amendation (mo)	Amendment			
		Control	Fertilizer	Sewage Sludge	Peat
0-5	0.5	66^{ab}	36^{ab}	196^{bc}	936^{de}
	15.0	58^{ab}	194^{bc}	255^{bc}	2596^{de}
	27.0	53^{ab}	127^{abc}	495^{cd}	1595^{de}
	39.0	45^{a}	119^{abc}	334^{bc}	2951^{e}
5-15 (15-25 in peat)	0.5	60^{abc}	60^{abc}	60^{abc}	320^{c}
	15.0	50^{ab}	110^{abc}	80^{abc}	150^{bc}
	27.0	40^{ab}	50^{ab}	140^{abc}	100^{abc}
	39.0	40^{ab}	30^{a}	40^{ab}	60^{abc}

[1] The 0-5 cm data was analyzed by a two-way MANOVA after ln Y transformation (MSE = 9976). Significant differences amongst means were determined by a method analogous to the Scheffé multiple contrasts for pairwise comparisons used for the ANOVA. The 5-15 cm data were analyzed by a two-way ANOVA after ln Y transformation (MSE = 138.70). Significant differences amongst the values were determined by Scheffé multiple contrasts for pairwise comparisons. Values for each depth, followed by the same letter(s) do not differ significantly ($p \leq 0.05$). All means are geometric.

Table 104. The effect of sampling depth on microbial activity (CO_2 ↑) in the variously amended oil sands tailings planted with slender wheatgrass[1].

Time after amendation (mo)	Amendment			
	Control	Fertilizer	Sewage Sludge	Peat
0.5	NS	NS	*	*
15.0	NS	NS	*	*
27.0	NS	*	*	*
39.0	*	*	*	*

---

[1] See Table 42 for explanation of statistical analysis.

NS - depths not significantly different ($p \leq 0.05$).

* - 0-5 cm CO_2 ↑ significantly greater ($p \leq 0.05$) than 5-15 cm (15-25 in the peat) CO_2 ↑ .

Table 105. Development of microbial biomass (mg C 100 g^{-1} dwt soil) in variously amended oil sands tailings planted with slender wheatgrass[1].

Soil depth (cm)	Time after amendation (mo)	Amendment			
		Control	Fertilizer	Sewage Sludge	Peat
0-5	0.5	3.85^{abc}	1.57^{a}	17.22^{bcde}	90.26^{ef}
	15.0	3.86^{abc}	11.91^{bcd}	33.96^{de}	241.00^{fg}
	27.0	4.66^{abc}	14.64^{bcde}	96.97^{e}	267.58^{fg}
	39.0	3.74^{ab}	15.74^{c}	77.78^{e}	486.11^{g}
5-15	0.5	4.73^{a}	2.77^{a}	2.77^{a}	22.67^{b}
	15.0	3.14^{a}	5.51^{ab}	7.83^{ab}	7.48^{ab}
	27.0	3.62^{a}	3.94^{a}	11.68^{ab}	11.51^{ab}
	39.0	3.91^{a}	2.97^{a}	5.96^{ab}	4.46^{a}

---

[1] Data analyzed by a two-way MANOVA after ln Y transformation (MSE = 11.65 and 4.80 for 0-5 cm and 5-15 cm depths respectively). Significant differences amongst means for each depth were determined by a method analogous to the Scheffé multiple contrasts for pairwise comparisons used for the ANOVA. Values for each depth, followed by the same letter(s) do not differ significantly ($p \leq 0.05$). All means are geometric.

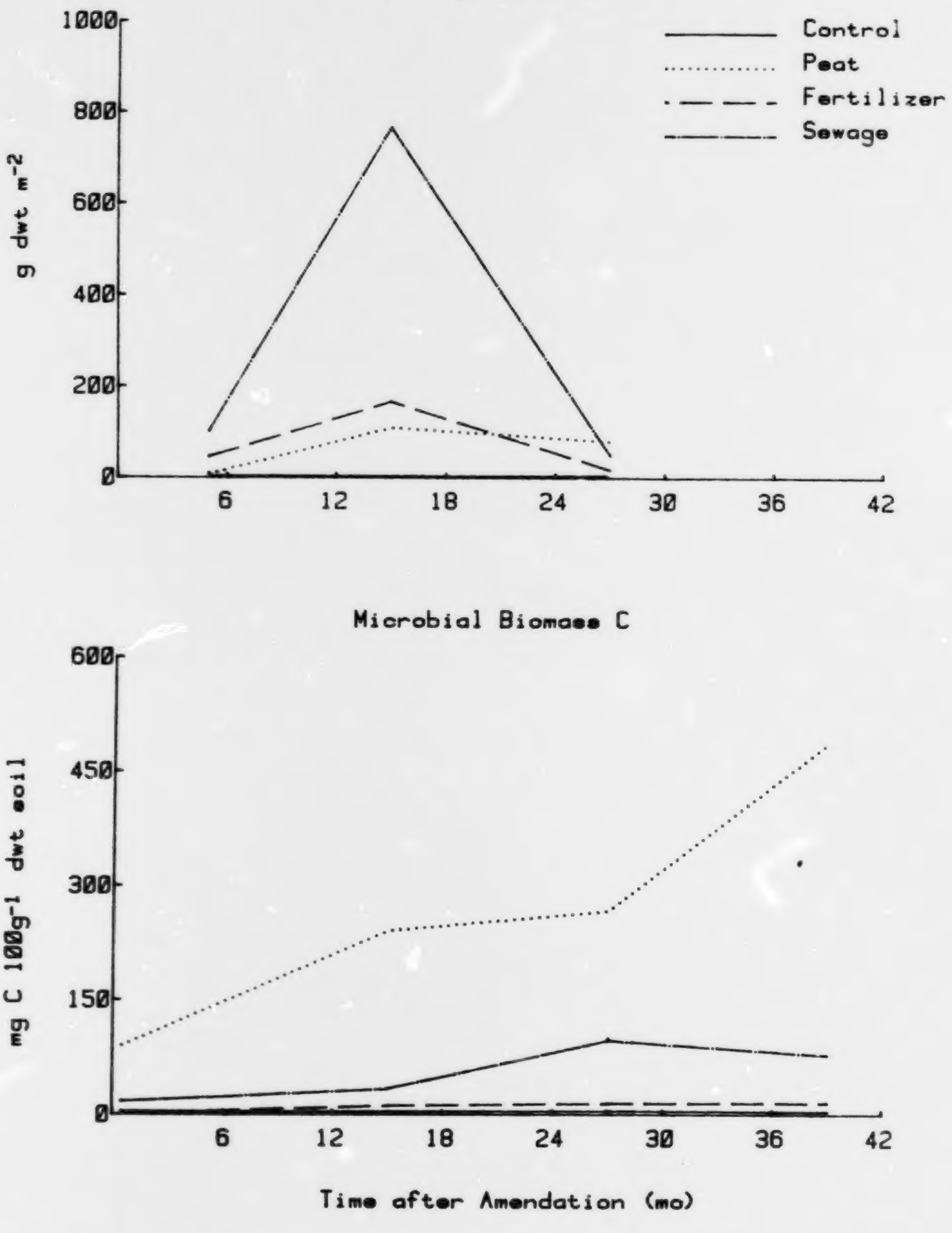

Fig. 17 Primary production and the development of microbial biomass in amended oil sands tailings planted with slender wheatgrass.

different from each other (peat > sewage sludge > fertilizer > control).

Amendation and plant growth effects on the microbial biomass C occurred only in the 0-5 cm deep soil. There were no obvious biomass C differences recorded amongst the various treatments in the 5-15 cm deep soil (Table 105). Only the control plots and fertilized plots at 0.5 mo demonstrated no significant depth effects on microbial biomass C - the other treatments (regardless of sampling time) harbored more microbial biomass in the surface than subsurface soil (Table 106).

Although it was hypothesized that litter input and root wts in the slender wheatgrass plots were related to microbial activity and biomass, there were no significant correlations amongst these variables (Table 107). More replications within each treatment might have yielded a more significant relationship between grass productivity and microbial biomass C. Significant relationships were, however, obtained between % loss on ignition and microbial activity and biomass C.

3.2.4.5 Comparison of microbial development in unvegetated plots and plots planted with grass or pine. Organic matter estimated in soil from the amended but unvegetated pathways, in soil from the plots planted with jack pine and in soil from the plots planted with slender wheatgrass suggested that the planting of slender wheatgrass resulted in significantly more organic matter accretion than observed for the other two treatments over the 39 mo term of the study (Table 108). This observation was true for all four treatments.

Microbial biomass C, like organic matter, also tended to be higher in the 0-5 cm soil from the grass plots than soil from the unvegetated pathways and the jack pine plots (Table 109, Fig. 18). This was not the case for the control plots where primary production was negligible. Unfortunately, variation in biomass C measurements were extremely high making it difficult to further discuss the role of different vegetation types in the development of microbial activity and biomass in reclaimed minespoils. More detailed research

Table 106. The effect of sampling depth on microbial biomass C in the variously amended oil sands tailings planted with slender wheatgrass[1].

Time after amendation (mo)	Amendment			
	Control	Fertilizer	Sewage Sludge	Peat
0.5	NS	NS	*	*
15.0	NS	*	*	*
27.0	NS	*	*	*
39.0	NS	*	*	*

---

[1] As a result of dependence between the 0-5 cm and 5-15 cm data, the effect of depth was determined by subtracting the 5-15 cm values from the 0-5 cm values and analyzing the resulting data using the model for the two-way ANOVA (i.e. microbial biomass C = $\overline{\mu}_{..}$ + (amendment effect) + (time effect) + error). The null hypothesis was that each treatment mean = 0 (i.e. no difference between depths). This method allows examination of each treatment in the event that not all treatments show an effect due to depth. NS - depths not significantly different ($p \leq 0.05$). * - 0-5 cm biomass C significantly greater than 5-15 cm (15-25 in the peat) biomass C ($p \leq 0.05$).

Table 107. Product moment correlation coefficients for loss on ignition, litter input, root wt, CO_2 ↑ and microbial biomass C data collected 27 and 39 mo after amendation of the oil sands tailings (slender wheatgrass plots).

	% loss on ignition	Grass litter input	Grass root wt	Microbial activity (CO)	Microbial biomass C
% loss on ignition	1	.049	.329	.727	.905*
Grass litter input		1	.551	.192	.244
Grass root wt			1	.368	.476
Microbial activity (CO ↑)				1	.832*
Microbial biomass C					1

*indicates significant correlation

Table 108. Effect of vegetation on % loss on ignition of the amended oil sands, 39 mo after planting[1].

Plant type	Amendment				Plant type $\overline{X}$
	Control	Fertilizer	Sewage Sludge	Peat	
Unvegetated	.26	.20	2.10	24.52	1.28^a
Slender wheatgrass	.38	.54	2.54	41.59	2.16^b
Jack pine	.27	.29	1.44	27.63	1.32^a
Amendment $\overline{X}$	$.30^a$	$.31^a$	1.97^b	30.43^c	

---

[1] Data analyzed by two-way ANOVA after ln Y transformation (MSE = .200). No significant interaction was observed, hence Scheffé multiple contrasts for pairwise comparisons were applied to amendment and plant type means. Values in the plant type $\overline{X}$ column or amendment $\overline{X}$ row, followed by the same letter do not differ significantly ($p \leq 0.05$). All means are geometric.

Table 109. Effect of vegetation on microbial biomass (mg C 100 g^{-1} dwt soil) in the amended oil sands tailings 39 mo after planting[1].

Plant type	Amendment			
	Control	Fertilizer	Sewage Sludge	Peat
Unvegetated	3.97^{ab}	3.62^{a}	23.60^{cde}	121.56^{fgh}
Slender wheatgrass	3.74^{a}	15.74^{bcd}	77.78^{efg}	486.11^{h}
Jack pine	8.57^{abc}	9.80^{abcd}	39.35^{def}	215.53^{gh}

---

[1] Data analyzed by two-way ANOVA after ln Y transformation (MSE = .281). Values followed by the same letter(s) do not differ significantly ($p \leq 0.05$) as determined by Scheffé multiple contrasts for pairwise comparisons. All means are geometric.

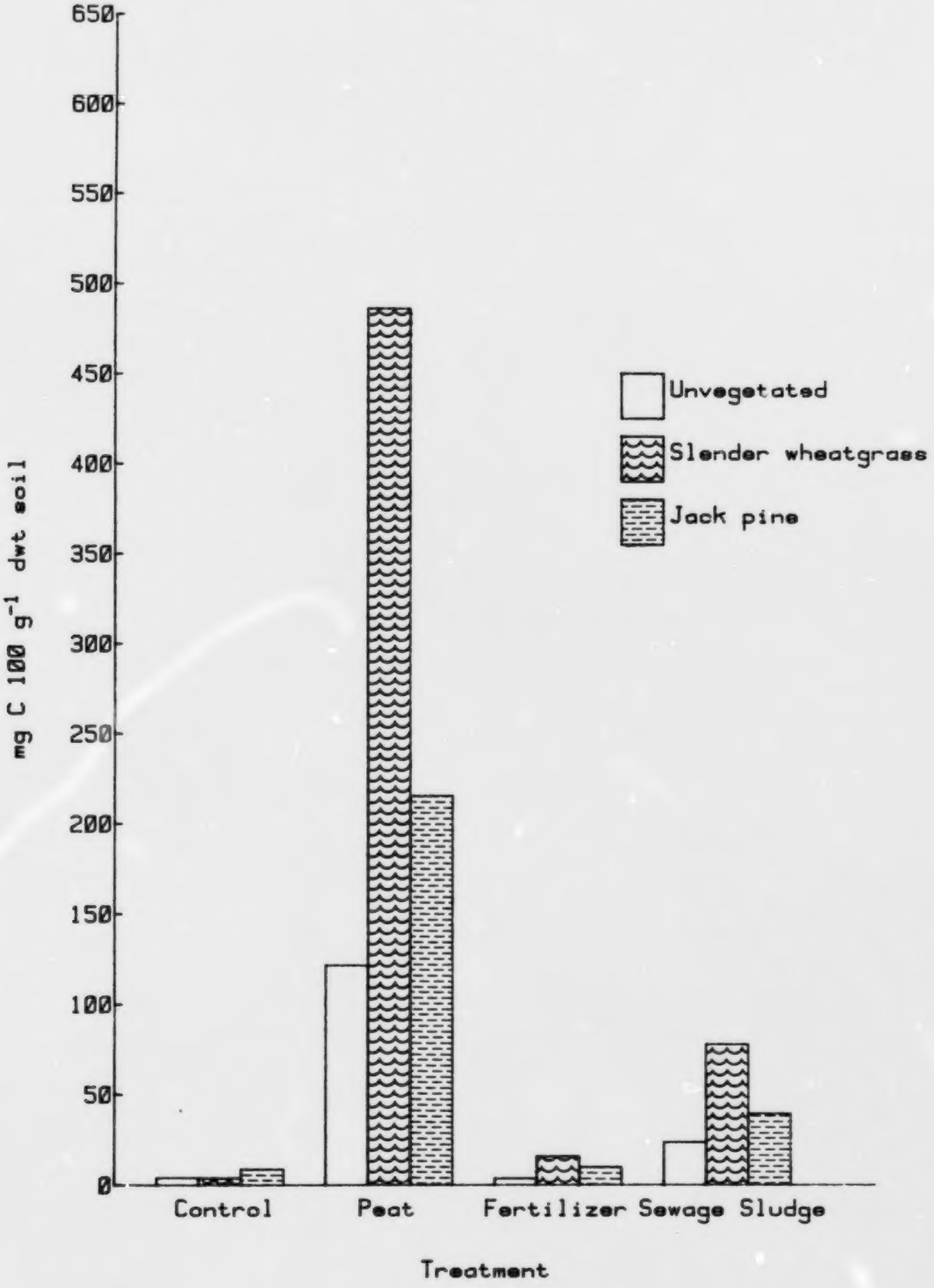

Fig. 18 Effect of vegetation on microbial biomass in the amended oil sands tailings 39 months after planting.

with more replication is required to elucidate the relationship between primary productivity and the microbial system.

3.2.4.6 Decomposition potential of amended and planted tailings sand. Cellulose filter paper placed in the slender wheatgrass plots for 12 mo lost more wt in the sewage sludge treatment than in the other three treatments (Table 110). After spending 24 mo in the field, the % wt remaining of filters in the fertilizer and sewage sludge amended plots was significantly less than the wt remaining in the untreated and peat treated plots. Only filters in the fertilized slender wheatgrass plots lost a significant amount of wt between the two sample times.

Decomposition of filters placed in the jack pine plots was not significantly influenced by amendation and jack pine growth after 12 or 24 mo in the field (Table 110).

As in the other decomposition studies where fir wood dowel was used as a test substrate, there was very little wt loss recorded for this substrate regardless of treatment, plant type or incubation time (Table 111).

With the exception of the sewage sludge treated plots, the ability of the fertilized, peat amended and unamended sand to decompose cellulose did not change significantly over the term of the study (Table 112). In the sludge treated slender wheatgrass plots the cellulose decomposition potential of the soil decreased between 16 and 40 mo after planting. At the termination of the study (40 mo) cellulose decomposition tended to be more rapid in the fertilized plots than in the other treatments.

The decay of slender wheatgrass leaves placed in the grass plots 28 mo after planting was not significantly influenced by amendation (Table 113). A significant wt loss occurred between the 6 and 12 mo incubation times for leaves in all treatments. Interestingly, the % wt remaining of leaves placed in the oil sands plots for 12 mo (i.e. 44-46%) was very similar to that obtained for leaves placed in the subalpine plots (44-48%) for the same length of time.

Table 110. Cellulose filter paper decomposition (expressed as % wt remaining) in amended sand tailings plots planted with slender wheatgrass and jack pine. Filter paper was placed in the plots 4 mo after planting[1].

Plant type	Time in the field (mo)	Amendment				Time $\bar{X}$
		Control	Fertilizer	Sewage Sludge	Peat	
Slender wheatgrass	12	99^b	68^b	10^a	87^b	
	24	90^b	29^a	4^a	69^b	
Jack pine	12	88	91	46	67	73^a
	24	67	62	34	56	55^a
	Amendment $\bar{X}$	78^a	77^a	40^a	62^a	

---

[1] Data analyzed by two-way ANOVA (MSE = 105 and 809 for slender wheatgrass and jack pine respectively). A significant interaction was observed for the slender wheatgrass data, hence Scheffé multiple contrasts for pairwise comparisons were applied to individual means. Values followed by the same letter do not differ significantly ($p \leq 0.05$).

Table 111. The % wt remaining of fir wood dowel placed in amended sand tailings plots 4 mo after planting slender wheatgrass and jack pine[1].

Plant type	Time in the field (mo)	Amendment				Time $\overline{X}$
		Control	Fertilizer	Sewage Sludge	Peat	
Slender wheatgrass	12	99	99	98	98	98.5^a
	24	98	99	98	99	98.5^a
	Amendment $\overline{X}$	98.5^a	99^a	98^a	98.5^a	
Jack pine	12	99	99	98	98	98.5^a
	24	98	99	99	98	98.5^a
	Amendment $\overline{X}$	98.5^a	99^a	98.5^a	98^a	

[1] Data analyzed by two-way ANOVA (MSE = .491 and .098 for slender wheatgrass and jack pine data respectively). Values presented in the table have been rounded off. No significant differences were observed.

Table 112. The decomposition of cellulose filter paper (expressed as % dwt remaining) after 12 mo in extracted oil sands plots planted with slender wheatgrass. Filters were placed in plots 4 mo and 28 mo after planting[1].

Time after planting (mo)	Amendment			
	Control	Fertilizer	Sewage Sludge	Peat
4	99.9^{c}	68.9^{bc}	6.4^{a}	90.5^{bc}
28	86.7^{bc}	36.8^{ab}	71.1^{bc}	80.5^{bc}

---

[1] Data analyzed by a two-way ANOVA after 2 x arcsin $\sqrt{p}$ transformation (MSE = 0.24). A significant interaction was observed, hence Scheffé multiple contrasts for pairwise comparisons were applied to individual means. Values followed by the same letter(s) do not differ significantly ($p \leq 0.05$). All means are geometric.

Table 113. Decomposition (expressed as % dry wt remaining) of slender wheatgrass leaves incubated in the amended oil sands tailings plots for 6 mo and 12 mo. Plots had been planted with slender wheatgrass for 28 mo prior to initiation of experiment[1].

Incubation time (mo)	Amendment				Time $\overline{X}$
	Control	Fertilizer	Sewage Sludge	Peat	
6	88	75	78	77	79^b
12	46	41	46	44	44^a
Amendment $\overline{X}$	67^a	58^a	62^a	60^a	

[1] Data analyzed by two-way ANOVA (MSE = 72.62) and Scheffé multiple contrasts for pairwise comparisons. Values in the time $\overline{X}$ column or amendment $\overline{X}$ row followed by the same letter do not differ significantly ($p \leq 0.05$).

A comparison of the % wt remaining for slender wheatgrass leaves, stems and filter papers after 12 mo decomposition is presented in Table 114 and Fig. 19. Neither leaf nor stem decay was affected by treatment, but leaves decayed much more rapidly than stems in all treatments. The results obtained for the filter paper were highly variable, but a significant difference in % wt remaining was observed between filters placed in the fertilized and untreated plots. Filters placed in the sewage sludge, peat and untreated plots lost approximately the same weight as the stems over the 12 mo experimental period (Fig. 19).

A multiple regression of % dry stem or leaf wt remaining = a + b (% loss on ignition of soil below the respective litter bags) + c (dead litter wt above and below the litter bags at retrieval) + d (root wts in 0-5 cm deep soil below the litter bag) + e (soil respiration in 0-5 cm deep soil below the litter bag) + f (microbial biomass C in 0-5 cm soil below the respective litter bags) demonstrated no significant regression line ($p = 0.60$, $F_{6,16} = .779$). Therefore, no significant relationship could be found between any of the relevant measured parameters and the decomposition of slender wheatgrass stem and leaf litter over the short term (12 mo).

Sainfoin, the test legume species planted in the oil sands plots, was the second most productive plant over the short term after slender wheatgrass (Visser et al., 1984). Hence it was considered important to study the decomposition of its leaves and stems and compare the wts remaining with those of slender wheatgrass stems and leaves after 12 mo decay. A three-way ANOVA was applied to the data and the results are presented in Tables 115 and 116. As in the three-way ANOVA conducted on the grass and clover decomposition data from the subalpine spoil, there was no significant three way interaction, but significant interactions were observed between litter type and plant type and between litter type and amendments (Table 115). To locate significant differences data were analyzed by the same methods as described for the grass and clover decomposition data collected for the subalpine spoil.

Table 114. The decomposition (expressed as % wt remaining after 12 mo in the field) of slender wheatgrass stems and leaves and cellulose filter paper placed in extracted oil sands plots 28 mo after planting slender wheatgrass.[1]

Litter type	Amendment				Litter type $\overline{X}$
	Control	Fertilizer	Sewage Sludge	Peat	
Leaves	46	50	46	44	46^{a}
Stems	81	75	66	72	73^{b}
Amendment $\overline{X}$	63^{a}	62^{a}	56^{a}	58^{a}	
Filter paper[2]	$84^{b} \pm 13.9$	$38^{a} \pm 20.3$	$70^{ab} \pm 23.0$	$75^{ab} \pm 25.8$	

[1] Leaf and stem data analyzed by two-way ANOVA (MSE = 157.4). Scheffé multiple contrasts for pairwise comparisons were applied to litter type means. Values in the litter type $\overline{X}$ column or amendment $\overline{X}$ row followed by the same letter do not differ significantly ($p \leq 0.05$).

[2] Filter paper data could not be included in the ANOVA due to considerable variations. Data was analyzed by multiple t-test based on the Behrens-Fisher t-distribution. Means followed by the same letter(s) do not differ significantly ($p \leq 0.05$). Standard deviations are also included.

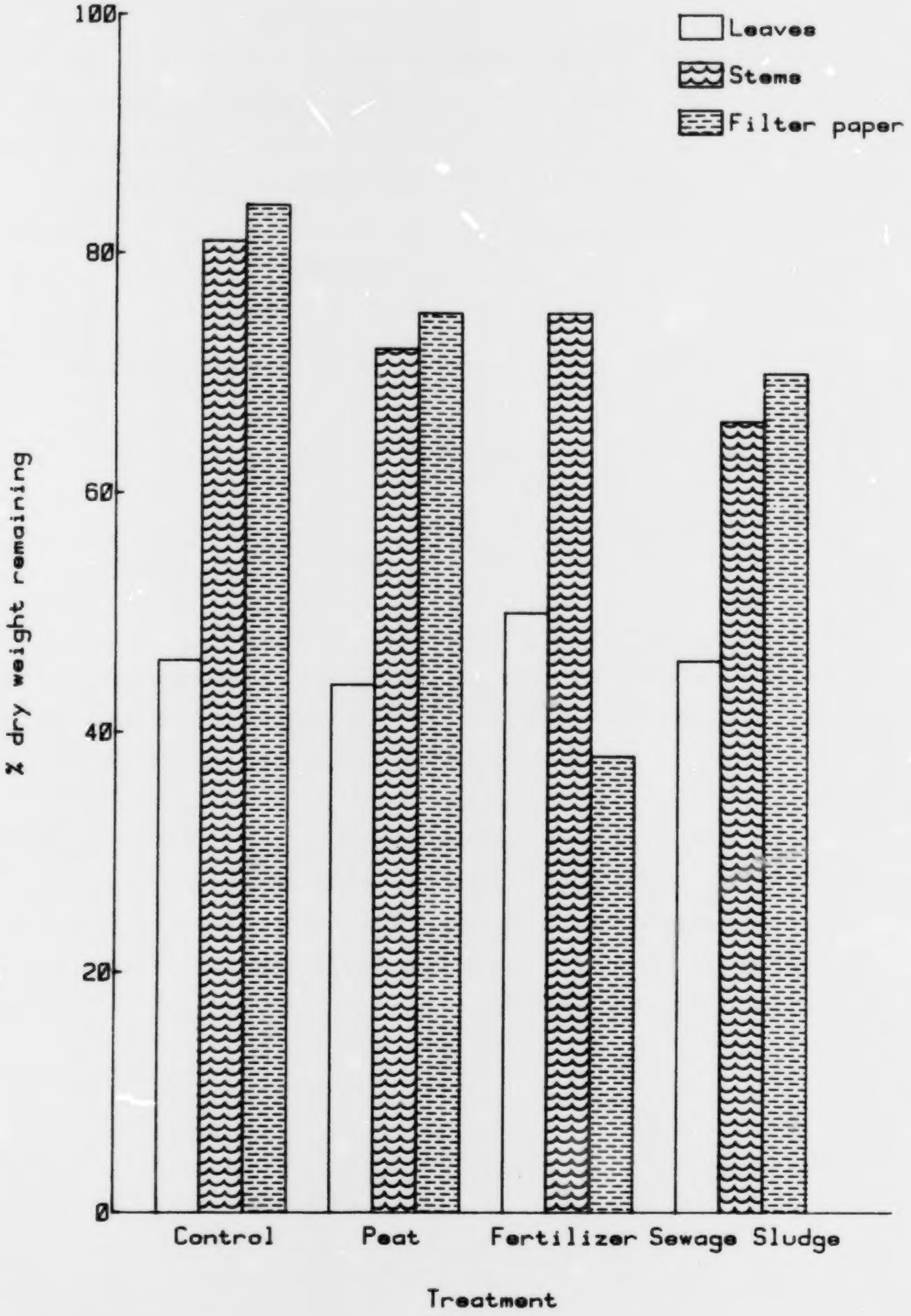

Fig. 19 The decomposition of slender wheatgrass leaves and stems and cellulose filter paper placed in amended oil sands tailings 28 months after planting slender wheatgrass.

Table 115. Three-way ANOVA table to test the effect of litter type (i.e. stems or leaves), plant type (i.e. slender wheatgrass or sainfoin) and amendment (i.e. control, fertilizer, sewage sludge and peat) on % dry litter remaining after 12 mo incubation in the oil sands tailings plots.

Source	SS	df	MS	F	prob.[1]
Litter type	6864.78	1	6864.78	93.21	***
Plant type	6.93	1	6.93	0.09	NS
Amendment	369.68	3	123.23	1.67	NS
Litter type x plant type	3550.23	1	3550.23	48.21	***
Litter type x amendment	623.98	3	207.99	2.82	*
Plant type x amendment	268.03	3	89.34	1.21	NS
Litter type x plant type x amendment	167.50	3	55.83	0.76	NS
Error	589.63	80	73.65		
Total	17742.76	95			

[1] * - significant at $p \leq 0.05$

*** - significant at $p \leq 0.001$

NS - not significant.

Table 116. The effect of amendation on the decomposition (expressed as % dry litter remaining after 12 mo incubation) of slender wheatgrass and sainfoin litter placed in the extracted oil sands plots 28 mo after planting (extension of data presented in Table 115)[1].

Treatment	Plant or litter type	Amendment			
		Control	Fertilizer	Sewage Sludge	Peat
% dry leaf wt remaining (litter type x plant type x amendment)	Sainfoin	58	59	60	51
	Slender wheatgrass	46	41	46	44
% dry stem wt remaining (litter type x plant type x amendment)	Sainfoin	61	67	57	62
	Slender wheatgrass	81	75	66	72
% dry sainfoin and grass leaf or stem wt remaining (litter type x amendment means)[1]	Leaves (sainfoin plus grass)	52^{ab}	50^{ab}	53^{ab}	47^{a}
	Stems (sainfoin plus grass)	71^{c}	70^{c}	61^{bc}	67^{c}

(cont'd)

Table 116. (cont'd).

Treatment	Plant or litter type	% dry wt remaining (summed)	
		Leaves	Stems
% dry leaf or stem wt remaining when summed over amendments (litter type x plant type means)[1]	Sainfoin	57^b	62^b
	Slender wheatgrass	44^a	73^c

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[1] Means tested by Scheffé multiple contrasts for pairwise comparisons. Values in each category followed by the same letter(s) do not differ significantly ($p \leq 0.05$).

Table 116 presents a summary of the results. The main conclusions which can be drawn from the data are:

i) the leaves of the grass and sainfoin combined decomposed faster than the grass and sainfoin stems in all but the sewage sludge treatment where no significant differences were recorded between the decay of leaves and stems.

ii) when the decay of sainfoin leaves and stems together was compared with the decay of grass leaves and stems, no differences were detected between the plant types. The decomposition of sainfoin litter (i.e. stems and leaves) was equally fast amongst the four treatments - (peat, sewage sludge, fertilizer, control) - a result also obtained for slender wheatgrass.

iii) slender wheatgrass leaves decayed more rapidly than sainfoin leaves, but sainfoin stems lost more wt than grass stems over the short term (12 mo). Sainfoin leaves and stems decayed equally fast; whereas slender wheatgrass stems were slower to decompose than the grass leaves.

3.2.4.7 N_2 fixation capacity of sainfoin. The N_2 (C_2H_2) fixation capacity of sainfoin at the conclusion of each of the first three growing seasons is presented in Table 117. After the first growing season, fixation potential was very low and no significant amendation effects were detected. N_2 (C_2H_2) fixation after 14 mo growth had increased but was highly variable so again no differences were recorded amongst the four treatments. At the conclusion of the third growing season however, sainfoin from the sludge treated tailing sand reduced significantly less C_2H_2 than sainfoin from the control, fertilized and peat amended plots.

No strong relationships were observed between N_2 fixation by sainfoin and soil NO_3-N, total soil N, shoot N, root N and shoot wt as determined by partial correlation coefficients (Table 118). Similar results were obtained for alsike clover in the subalpine spoil.

Table 117. N_2 (C_2H_2) fixation capacity (nmoles g^{-1} dry root hr^{-1}) of sainfoin in extracted oil sand tank plots[1].

Treatment	Time after planting		
	3 mo	14 mo	26 mo
Control	3.8^a	1032.0^a	151.5^a
Fertilizer	0.9^a	776.3^a	68.0^a
Sewage Sludge	1.5^a	4.7^a	2.4^b
Peat	5.6^a	233.0^a	125.1^a
MSE	32.52	9.81	3.04

---

[1] Data was analyzed by a one-way ANOVA and Scheffé multiple contrasts for pairwise comparisons. The 14 and 26 mo data required a ln (Y + 1) and ln transformation respectively. Values in each column followed by the same letter do not differ significantly ($p \leq 0.05$). The 14 and 26 mo means are geometric.

Table 118. Partial correlation coefficients for determining the relationships amongst shoot wt, root wt, shoot N, total soil N, soil NO_3-N and N_2 (C_2H_2) fixation capacity for sainfoin after 1978 growing season.

	Shoot wt (g)	Root wt (g)	Shoot N (%)	Total Soil N (%)	Soil NO_3-N (µg/g dry soil)	N_2 (C_2H_2) fixation (nmoles/g root/hr)
Shoot wt (g)		.86	.41	.40	.18	-.29
Root wt (g)			.42	.67	.33	-.32
Shoot N (%)				.41	.38	-.39
Total soil N (%)					.86	-.17
Soil NO_3-N (µg/g dry soil)						-.17
N_2 (C_2H_2) fixation (nmoles/g root/hr)						

4. CONCLUSIONS AND DISCUSSION

4.1. FIELD STUDY

4.1.1 Microbial Numbers and Hyphal Lengths

With the exception of the grassland sites where numbers of bacteria in the 0-5 cm disturbed soil were greater than in the undisturbed topsoil and actinomycete numbers were as high in the subsurface (5-15 cm) minespoil as in the undisturbed grassland, there was a general decrease in bacterial and actinomycete counts and hyphal lengths as a result of mining at the various study sites. Many of the decreases for the individual microbial components were especially evident when results from the surface undisturbed soil were compared to those from the disturbed soil. Differences in the microbial numbers and hyphal lengths were not as obvious when the undisturbed subsurface (mineral) soil was compared with the mined soil. No doubt, the decreases in the microbial populations are a result of the removal of organic material from the soil during the mining process. The A_h horizon in the grasslands and the FH layers in the subalpine and oil sands forests are a major source of energy and nutrients for the microbes; also, many of the plant roots and their associated microorganisms are located in these layers. It therefore seems obvious that by stripping the soil of its organic constituents, a decrease in the microbial populations would automatically result. Data presented by Visser et al. (1984) show that, particularly in the two forest sites, mining resulted in the almost complete removal of the biologically available organic C.

Wilson (1965) and Müller (1973) working with acidic coal mine spoils also observed that bacterial and actinomycete numbers were lower in barren minespoils than in vegetated or undisturbed soils. However, both researchers came to the conclusion that the presence of vegetation was one of the most important factors in re-establishing the soil microflora. Input of organic matter, from the primary producers, through dead leaves and roots and root exudates, was probably the basis for stimulating microbial growth in the revegetated minespoils they studied. Neither Wilson (1965) nor Müller (1973) measured a significant effect of mining and revegetation on the fungi. Their data, however, are difficult to interpret since soil dilution plates were used in their studies. As mentioned in the introduction, fungal plate counts tend to be nothing more than counts of inactive spores. In the present study, the effects of mining on the fungal component were determined by a direct observation technique, and it is obvious from the results obtained that mining significantly decreased the length of fungal hyphae (both total and that with cell contents) at two of the study sites.

Fresquez and Lindemann (1982), who studied the microbiological parameters in what appeared to be a similar spoil to the grassland spoil in this investigation, recorded significantly fewer bacteria in the barren spoil than in the undisturbed soil. As mentioned previously, bacterial numbers in the surface soil layers at the grassland site were significantly greater in the minespoil than undisturbed topsoil. One of the reasons for the discrepancy in results between the present study and that of Fresquez and Lindemann (1982) may be the differences in the ages of the spoils in the two studies. The spoil in this study was a result of mining in the late 1940's; the time elapsed since then may have given the bacterial populations a chance to increase. Also reclamation attempts of this spoil in the 1970's resulted in the luxurious growth of Kochia scoparia (L.) Schrad. The stimulation of bacterial growth may have occurred in the rhizosphere of this plant, thereby significantly increasing the numbers. Wilson (1965) showed that numbers of bacteria in the rhizosphere and on the surface of the plant roots in a vegetated spoil were greater than those on roots in a non-vegetated spoil.

In comparing the effects of soil disturbance on various groups of soil microorganisms quantified in this investigation, it must be remembered that different methods were used for each group (i.e. dilution plating for actinomycetes and bacteria, direct measurement for fungi). Because of this, direct comparisons amongst the groups may be misleading. Nevertheless, it appears that of the three microbial groups studied, the bacteria were the most capable of surviving and growing under the low nutrient regimes and environmental extremes found in mined soils, while the fungi were the most sensitive.

4.1.2 Bacterial Community Composition

When the bacterial number data are viewed in conjunction with the data on the bacterial groups isolated from the grassland, and subalpine undisturbed and disturbed sites, it can be seen that mining resulted in a significant increase in the proportion of the bacterial community occupied by the coryneform-type bacteria. Prior to disturbance of the grassland, Bacillus and the unpigmented Gram negative rods were common in the soil, while the bacterial community in the undisturbed subalpine site was dominated by Cytophaga, Flavobacterium (organic soil only) and the unpigmented Gram negative bacteria. The unpigmented Gram negative bacteria were also the most frequently isolated bacteria from the unmined oil sands site, along with the coryneforms.

Nelson (1978a) found that when growing selected isolates of arctic soil bacteria in continuous culture under low temperature and limiting nutrient levels, a Bacillus sp. seemed the least adapted to these conditions while Arthrobacter (a coryneform-type bacterium) was capable of maintaining a viable population under this regime. She also observed (Nelson, 1978b) that Arthrobacter was resistant to freeze-thaw damage. The low nutrient and extreme temperature conditions often found in minespoils may give a competitive advantage to the coryneform group of bacteria over the possibly more susceptible Bacillus spp. and non-pigmented Gram-negative bacteria. The disappearance of Cytophaga as a result of mining at the subalpine site, may have been due to lack of moisture and organic matter in the minespoil. Cytophaga spp. are commonly isolated from very wet soils and are significant for their ability to degrade relatively decay resistant substrates such as cellulose and chitin (Christensen, 1977). If Cytophaga spp. in the organic mat of the undisturbed spruce-fir forest at the subalpine site were active in decomposing the chitinous walls of the large fungal standing crop measured in that soil, the loss of this mycelial substrate through mining would undoubtedly decrease the frequency of *Cytophaga*. It is interesting to note that storage of the bacteria in semi-solid peptone-yeast extract agar at 5°C for six to eight months adversely affected the viability of isolates from the undisturbed soils (particularly the subalpine site) but not the viability of bacteria from the minespoils. This phenomenon may be an indication that bacteria from undisturbed systems are more specialized in terms of their nutrient, temperature and moisture requirements making them more sensitive to changing environmental conditions than are the less specialized, presumably less sensitive bacteria which survived in the disturbed soils.

4.1.3 Fungal Community Composition

Studies which have dealt with the effects of mining on the fungal genera have shown that in general the diversity of fungal taxa in barren minespoils is less than that in undisturbed soil (Lawrey, 1977; Fresquez and Lindemann, 1982). However, these investigations have demonstrated that the incorporation of organic amendments (hay, sewage sludge) or the evolution of a vegetative cover on the minespoil will increase the fungal taxa diversity, inferring that it is the lack of organic matter which limits the number of fungal genera in minespoils.

In the present study, each of the three minespoils was different in terms of its fungal generic distribution. At the grassland site, the spoil contained a large number of fungal taxa with the dominant forms being *Cladosporium* spp., *Alternaria* spp., *Penicillium* spp., sterile dark forms and yeasts. *Cladosporium*, *Alternaria* and yeasts are widely distributed and commonly colonize leaf litter and soil (see reviews of first two genera in Domsch et al., 1980). They are frequently isolated from the atmosphere, hence would be the first group of organisms to have the opportunity to colonize the surface of recently mined soil. *Penicillium* spp., a ubiquitous group of saprophytes which have the capability to utilize a wide variety of substrates and can survive such extreme environmental conditions as low water potentials and high temperatures (see review of this taxon in Domsch *et al*., 1980), were the most frequently isolated fungi from the subsurface minespoil. The number of plated soil particles which yielded fungi was approximately the same for the undisturbed and disturbed sites, indicating that the grassland minespoil was well-colonized.

The diversity of fungal taxa isolated from the disturbed subalpine soil was not as great as that observed in the undisturbed spoil. Also, with the exception of *Candida* spp. and *Chrysosporium* spp. many of the fungi in the minespoil occurred with a very low frequency. Lack of organic matter, moisture and nutrient stress in the minespoil would be the major factors explaining these data. Interestingly, mining at the grassland site resulted in a significant decrease in *Chrysosporium-Pseudogymnoascus*, but at the subalpine site, the occurrence of *Chrysosporium* (mainly *C. pannorum*) increased significantly after mining. The increased frequency of *C. pannorum* in the subalpine minespoil, may be a result of the rise in soil pH after mining (pH 4.2-4.5 in undisturbed soil and 7.0-7.3 in mined soil) since this organism appears to prefer soils of a neutral pH (see review of this species in Domsch et al., 1980). Also laboratory studies of substrate utilization by this fungus have demonstrated that this organism is capable of utilizing a wide variety of substrates (starch, pectin, cellulose, chitin, tannins). This capability may give *Chrysosporium pannorum* a competitive advantage over other fungi, hence its frequency in the nutrient impoverished conditions of the spoil. Only one other study has recorded a *Chrysosporium* sp. from minespoils. Evans (1971) isolated a thermophilous *Chrysosporium* from coal spoil tips in England. The preference of *Chrysosporium* for a neutral pH would explain why this fungus has not been isolated from acidic minespoils in the eastern U.S.

In contrast to the grassland spoil, less than 50% of the subalpine minespoil particles yielded fungi. The older age of the grasslands spoil may have allowed the fungi to colonize that spoil to a greater extent than the subalpine spoil. The subalpine spoil had been spread immediately prior to sampling. It should be noted that the fungi isolated from the stockpiled regolith were slightly different from those isolated from the disturbed plot (i.e. in addition to *Chrysosporium*, *Phialophora* sp. and *Acremonium* spp. were dominant forms). Also the number of soil particles yielding fungi was not substantially different from that observed for the undisturbed soils. This may infer that over the period of stockpiling, the fungal growth within the stockpiled soil decreases, while the fungal community shifts to one dominated by *Chrysosporium pannorum* and the yeast, *Candida*.

The harsh methods used to remove oil from the oil sands results in a fine-grained sand (86% of the grains fall in the 125-500 µm size range) which is devoid of organic matter and nutrients, and mycologically sterile. Fungal isolations made from the extracted sand, demonstrated that only the sand in contact with the atmosphere was colonized to any extent by fungi - these fungi consisting mainly of *Cladosporium* spp. and yeasts, organisms which are commonly transported through the atmosphere as mentioned previously. The poor fungal colonization of the extracted sand was further substantiated by the low number of plated particles which yielded fungi. It is obvious from these results that a highly organic amendment would be required to re-establish the fungal community in the sand tailings to a level where it would eventually attain the diversity observed in the undisturbed soils.

In general, the fungal isolation data demonstrated that the mining procedures and the age of the spoil could have a significant effect on the fungal community and its growth in the spoil. It appears that in spoils where the indigenous fungi are lacking or have been completely destroyed during mining, fungi common in the atmosphere (*Cladosporium*, *Alternaria* and yeasts) are the first colonizers, particularly in the surface minespoil.

It can be concluded from the microbial population data, that the minespoils at the three study sites exhibited a lower bacterial and actinomycete count and less fungal hyphae than that measured in nearby undisturbed areas. One exception to this was the grassland site where numbers of bacteria were greater in the disturbed than undisturbed soil. Also mining resulted in major shifts in the structure of both the bacterial and fungal communities. After mining, the bacterial community in both the grassland and subalpine areas was dominated by coryneforms, while the fungi occurring with the greatest frequency were organisms common in the atmosphere or in the case of the subalpine spoil, Chrysosporium and Candida.

4.1.4 Soil Respiration, Microbial Biomass C and ATP

Although the microbial population data infers that mining results in reduced microbial activity, plate counts and hyphal length measurements give very little indication of the activity of the isolated organisms when in the soil. Bacterial and actinomycete plate counts can include both active, vegetative cells and inactive spores, while the presence of protoplasm in fungal hyphae at the time of sampling does not necessarily mean the mycelium is active, but rather has a potential for being active. Also, the type of media used to isolate bacteria or actinomycetes may select only a certain proportion of the community, while major difficulties are encountered in the separation of mycelium from the soil (particularly in highly organic or clayey soils). As a result, plate counts and hyphal length measurements should be coupled with soil respiration or ATP measurements in an effort to more accurately estimate the level of activity exhibited by both isolated and nonisolated organisms.

The measures of microbial activity incorporated in this investigation included soil respiration ($CO_2\uparrow$), microbial biomass C and ATP. With the exception of the oil sands site, where no significant differences in CO_2 efflux were encountered between undisturbed and disturbed soil, all three parameters were significantly lower in the minespoil than in the soil from the adjacent undisturbed areas. Plate counts of bacteria and actinomycetes and fungal hyphal measurements supported these results. No doubt the decrease in microbial activity in the mined soil was mainly due to the lack of an organically rich surface layer (with its associated plant component, more favorable nutrient levels and more active microbial system), although the high Na levels in the grassland spoil may also have contributed to inhibiting microbial activity. Malik and Azam (1980) have reported a decrease in soil microbial biomass with increasing salinity. Hedrick and Wilson (1956) also observed a lower rate of CO_2 efflux in strip-mined spoils than in undisturbed soils and attributed this mainly to a lack of sufficient N in the spoil. Lawrey (1977b) recorded lower respiratory activity in mined soils than in undisturbed soils and considered that the high levels of trace metals made available by the low soil pH contributed to the reduced microbial activity of the strip-mined soils. Recently, Stroo and Jencks (1982) concluded from their study on enzyme activity and respiration in minesoils at various stages of reclamation, that as organic matter and N accumulated in a spoil, microbial activity would slowly recover.

ATP measurements have been considered by Hersman and Temple (1979) as one of the best and most rapid methods for determining biological activity in minespoils. Their results (Hersman and Temple, 1978), like those obtained in this study, demonstrated that ATP levels in undisturbed soils were higher than those in minespoils. If one has access to the equipment required for assaying ATP, this method may be rapid, but in the present study, ATP data was highly variable. Also, ATP in the extracted oil sand was so low that it was not measureable although some microbial biomass was measured in these samples using the physiological method of Anderson and Domsch (1978). Unsuitable ATP extraction techniques and the analysis of small quantities of soil (1 g in this study) probably contributed to the variation observed.

4.1.5 Comparison of Methods for Estimating Microbial Biomass C

Because microbial biomass C, ATP, bacterial and actinomycete numbers and fungal lengths are all quantitative measurements of microbial material in a soil, it was decided to compare them via Pearson product moment correlations. Prior to performing the correlations, all quantitative microbial estimates were converted to mg microbial biomass C 100 g^{-1} dry soil. To achieve this, the conversion factors used by Domsch et al. (1979) in their study comparing methods for soil microbial population and biomass studies, were employed. Correlation coefficients for data from the oil sands site could not be calculated because of missing data and the presence of zeros. For the grassland disturbed and undisturbed soil, the direct observation method (which included dilution plate counts of bacteria and actinomycetes and total hyphal lengths) had correlation coefficients of -0.53 and 0.37 with the microbial biomass C (physiological technique) and ATP techniques repsectively. A correlation of 0.16 was found between the ATP and microbial biomass C measurements. In contrast to the poor relationship amongst the techniques when applied to the grassland soil, correlation coefficients calculated for the subalpine soil data demonstrated a much closer relationship (direct observation method had correlation coefficients of 0.84 and 0.97 with the physiological and ATP techniques, respectively while ATP and the physiological techniques had a correlation coefficient of 0.81). Since a small number (12) of samples was used to calculate each correlation matrix only coefficients greater than 0.85 should be regarded as meaningfully significant. Some of the possible reasons for the differences in biomass C correlations between the grassland and subalpine soils and the general lack of a relationship amongst biomass values obtained by the different methods are:

1. as mentioned previously, dilution plates may be selective for a certain component of the bacterial community and therefore may underestimate the bacteria present in a particular soil. They can also overestimate the bacterial population in the vegetative phase if the bacteria are present mainly as spores. Actinomycete biomass values are probably underestimated by the dilution plate technique since they have a filamentous growth form.

2. soils high in clay, such as the grassland minespoil, may make it difficult to separate the fungal hyphae from the soil. In this study, the presence of clay in the agar films, was

responsible for a considerable amount of masking making it difficult to recognize the hyphae and therefore leading to underestimates of the fungal biomass. Masking by clay particles was not a problem in the subalpine soils, hence the hyphal lengths measured in these soils would be more accurate than those measured in the clayey, fine-textured grassland soil. The hyphae constituted approximately 90-95% of the bacterial, fungal biomass C in both the subalpine and grassland soils. Therefore an underestimate of the biomass C in the fungal component would severely affect correlations between biomass calculated from direct measurements and biomass measured by the physiological technique. On the other hand, total hyphal lengths were converted to biomass C prior to calculating the correlation coefficients. Slow decomposition rates of dead hyphae (which would be included in the total hyphae category) could result in an overestimate of the active fungal biomass calculated from direct measurements.

3. dilution plate counts of bacteria and agar film estimates of hyphae, when converted to biomass C, may be subject to major errors due to the inaccuracies in the conversion factors (e.g. cell diameter, density of cells, moisture content of cells). Van Veen and Paul (1979) pointed out some of the problems associated with the conversion factors necessary to convert bacterial counts and hyphal lengths to biomass.

4. ATP values can be underestimates as a result of inefficient extraction from clayey or highly organic soils or due to the high quench of ATP by the soils under investigation. These factors may have affected the ATP levels measured in the grassland soils and the subalpine minespoil since microbial biomass estimated from the direct observation and ATP methods were very different in these particular soils. Because the physical/chemical properties of soils can vary considerably, preliminary research to determine the most efficient extraction techniques and the ATP quenching properties of the soil extracts would be required to improve the accuracy of ATP estimates for determining microbial biomass C in different soil types. In addition, Domsch et al. (1979) emphasized that ATP assays

are difficult to convert to microbial biomass, and therefore the conversion factors are, at best, very crude. Greaves et al. (1973) in a study dealing with the relationship between microbial populations and ATP in a basin peat observed that bacteria in peat demonstrated considerable variation in their ATP contents and concluded that ATP determinations could not be used as biomass measurements. ATP determinations could, however, provide useful information on the metabolic state of microbial populations.

5. direct observation, cultural methods and ATP assays are generally performed on very small quantities of soil (usually less than 5 g). Because of the heterogeneous distribution of soil microorganisms this can lead to great variability in biomass values obtained on replicate samples by these techniques. Studies to determine optimum sample size and soil dilution would aid in decreasing this variation.

For these reasons it appears that the cultural methods, direct observations and ATP assays have major weaknesses if one wants to extrapolate the results obtained from these techniques to microbial biomass. It is the belief of the authors that the more direct physiological technique developed by Anderson and Domsch (1978) is a much more efficient and accurate method for estimating the microbial biomass C in the vastly different minespoils and soil types encountered in the present study. This method has the advantage of rapidly providing reproducible biomass values, which because of the large sample size utilized (usually 100 g/sample) exhibit much less variability than that calculated from the techniques mentioned previously. The physiological technique also allows one to measure biomass under standard temperature, moisture and substrate quality (optimum quantities of glucose are added to each soil) conditions, thereby substantially reducing the variability resulting from these three variables. This is particularly important if one is interested in comparing soils with very different chemical/physical properties. However, it should be noted that this technique is based on the conversion of 1 ml CO_2 to 40 mg microbial biomass C - a factor based primarily on biomass estimates obtained for agricultural soils. Therefore it may not be precisely applicable to other soil types. More research is required to determine the conversion factors for soils other than agricultural soils. Nevertheless, this method has proved to be quick, efficient and sensitive enough to measure small shifts in microbial biomass and is, therefore recommended for future studies particularly with clayey soils. Microbial activity would probably be most easily and accurately determined by measuring CO_2 efflux or O_2 uptake on reasonably large soil samples. Where detailed qualitative and quantitative information is required for the individual bacterial and fungal components of the biomass, one must still resort to cultural and direct observation methods.

4.1.6 Asymbiotic N_2 Fixation

Since N is generally accepted to be lacking in minespoils, N_2 fixation by heterotrophic bacteria could be a means of increasing N levels in the spoils. Data collected in this study, however, demonstrated that the grassland minespoil exhibited no N_2 (C_2H_2) fixation potential, while the subalpine minespoil fixed negligible quantities of N under the conditions laid down in this investigation. One of the major factors controlling asymbiotic N_2 fixation is an adequate supply of C to provide energy for N_2 fixing bacteria. The lack of easily available C (in the form of organic amendments or root exudates and plant litter) in minespoils would severely limit their N_2 fixation potential. Fresquez and Lindemann (1982) recorded no Azotobacter (an asymbiotic N_2 fixing bacterium) in the coal minespoils they studied, but amendation of the minespoil with alfalfa hay, fertilizer and topsoil significantly increased Azotobacter levels particularly in the rhizosphere of their test plant, Atriplex canescens. These results emphasize the importance of an adequate C supply for the growth of the asymbiotic N_2 fixing bacteria. Also, Knowles (1977) drew attention to the fact that the rhizoplane-rhizosphere region of plants (particularly in plants which are highly productive) appears to have a high potential for N_2 fixation since many readily available C substrates (sloughed roots, root exudates) occur there. Therefore, if the input of N through N_2 fixation is to be considered important for soils disturbed by mining, the application of a highly organic amendment and/or the planting of highly productive plant species would be a necessary requirement.

Skeffington and Bradshaw (1980) have suggested that the rates of N_2 fixation by free-living bacteria in china clay wastes are so low that their contribution to N accumulation in this particular spoil is probably negligible. One reason why this may be so is the lack of efficiency exhibited by the bacteria when fixing N. Gray and Williams (1971) presented an example where, on the basis of a requirement of 1 g of sugar to fix 5-20 mg N, Azotobacter would require 1122 kg of organic matter per year to fix 5-9 kg N/ha. This would imply a very high cell turnover rate, which according to these authors would be impossible due to the lack of available organic substrate for growth. Although Knowles (1977) has suggested that efficiencies of N_2 fixation in the soil may be 2-3 times greater than that suggested above, it would still seem that soil N accumulation by asymbiotic bacteria (particularly in minespoils) would be almost negligible.

The undisturbed soils, which contained more microbiologically available organic matter than their respective minespoils, had surprisingly low N_2 fixation potentials. This was especially true for the undisturbed grassland samples. Paul et al. (1971) in their studies on N_2 fixation in virgin grasslands in Saskatchewan, obtained similar results. Like Skeffington and Bradshaw (1980), they considered N inputs via rain and nodulated plants to be more important contributors of N. The low asymbiotic N_2 fixation by the soils they were studying was believed to be because the virgin grassland systems are in equilibrium, so N-loss mechanisms play a minor role in the system and N input requirements are, therefore, low. This theory may also apply to the grassland soil investigated in this study.

4.1.7 Decomposition Processes

The evolution of a self-maintaining vegetative cover on minespoils is ultimately dependent on the establishment of the decomposition and mineralization processes, since it is these processes which will ensure a continual nutrient supply to the primary producers. In the present investigation it was hoped that the decay rates of the standard substrates, cellulose filter paper and fir wood dowel, in both undisturbed and disturbed systems would provide comparative data of the effects of mining on the decomposition of relatively easily degradable (cellulose) and recalcitrant (wood) C resources.

4.1.7.1 Standard substrates (filter paper and wood dowel). The use of a woody substrate proved to be a poor indicator of the effects of mining on the decay of a lignified substrate, since very little weight loss occurred at either the grassland or subalpine sites over the course of the study. No differences were, therefore, detected between undisturbed and disturbed sites. Bunnell et al. (1977a, b) found that when modelling the effects of various variables on microbial respiration and substrate weight loss, the initial changes occurring in substrate weight resulted from microbial respiration rates which were 'chemical specific' and independently influenced by temperature and moisture levels. It is well recognized that woody substrates are comprised mainly of cellulose and lignin and are notorious for their high "recalcitrant C" and low N levels (the wood in this study contained .03% N). Hence, a nutrient-poor and decay resistant substrate such as this would provide very few readily available nutrients for microbial growth and therefore decompose very slowly. In an effort to remedy the high C/N ratio, wood dowel was amended with ammonium nitrate, but this did not hasten its decomposition. It is possible that much of the added N leached out of the wood before the microorganisms could utilize it or that the presence of a high level of ammonium N inhibited microbial growth. Kowalenko et al. (1978) recorded a reduction of soil microbial activity in the presence of N fertilization and attributed their results mainly to the lowering of soil pH. Other studies (Pavlica et al. 1977; Setua and Samaddar, 1980) have revealed that ammonia can behave as a volatile inhibitor of fungal spore germination. There is also a probability that the decay resistant quality of the C in lignified substrates would control the decomposition rate of wood to a greater degree than would the levels of other nutrients. Also, the very dry conditions (total annual moisture = 335 mm) and the extremes in temperature occurring at the grassland site are not conducive to rapid microbial growth and turnover. Low soil temperatures, characteristic of soils such as those in the subalpine site, have also been regarded as a major factor in the inhibition of the decay process (Moore, 1981).

In contrast to the wood dowel, decomposition of the cellulosic filter paper proceeded at a more rapid rate. Short term differences in cellulose decomposition were not detected at any of the three sites, but over the long term (i.e. 24-33 mo) filters placed in the grassland and subalpine study areas exhibited a faster decay rate in the disturbed than undisturbed plots. A possible explanation for the results obtained at the grassland site, is that heavy spring rains (which occurred approx. 20-24 mo after the initiation of the study) resulted in increased runoff caused by the high sodium adsorption ratio of the spoil - a factor which can restrict water infiltration into the soil (Power et al., 1978). The runoff embedded and sometimes buried the filters in the spoil resulting in ameliorated microenvironmental conditions around the filters with moisture conditions being greatly improved. Once buried, the filters decayed rapidly so that 44 mo after the initiation of the study, virtually all the cellulose had been utilized.

Filters placed in the undisturbed grassland, however, were not readily incorporated into the soil matrix. The erosion observed in the disturbed plot was absent in the undisturbed plot and the grassland vegetation would occasionally elevate the filters above the soil surface. Therefore, the filters were more subject to moisture and temperature extremes resulting in reduced decomposition. Similar observations were made at the subalpine study area. Again filters placed in the minespoil were eventually buried, while those in the undisturbed forest remained on the surface for the full term of the study. The incorporation of the filters into the forest floor appeared to be largely dependent on the growth of the feather moss (Hylocomium splendens) over the bag containing the filter. Once overgrown by the moss, the ameliorated moisture and temperature conditions would probably accelerate decay. The results obtained in this study imply that the instability of the minespoil can greatly influence the decomposition of a substrate by increasing its chances of becoming buried. Burial can protect the decomposing substrate from temperature and moisture extremes - variables which are major controlling factors affecting decay rates.

The rapid decomposition of the disturbed site filters, once buried, is interesting when viewed in conjunction with the microbial activity and biomass data discussed earlier. Although CO_2 efflux and microbial biomass C measurements were significantly lower in the disturbed than undisturbed soils from both the grassland and subalpine sites, cellulose degradation nevertheless, proceeded rapidly in the disturbed soil. One explanation for this phenomenon is that the very low available C status in the minespoils affected the microbial biomass, but once an easily available C source such as cellulose was added to such an impoverished system, the soil microorganisms (both in active and resting phases) were capable of rapidly utilizing it. Laboratory studies have confirmed that many of the fungi isolated from the disturbed soil produce cellulases (e.g. Chrysosporium-Pseudogymnoascus, Fusarium, Alternaria). Therefore, the potential for cellulose degradation was present in the grassland and subalpine minespoils. In fact, it is possible that due to the lack of available C in disturbed soils, decomposition may be more rapid than in undisturbed soils where other forms of C are available. Although both the filter paper and the minespoil generally lack N, amendation of the filters with ammonium nitrate did not accelerate their decay, presumably for the same reasons mentioned previously for the wood dowel. Also, the presence of a readily available C source such as cellulose may regulate decomposition in minespoils to a greater degree than mineral nutrients, which were present in low quantities in the two spoil types.

The relationship between decomposition of cellulosic filter paper and that of natural litter is questionable. Natural litter and filters placed out in the undisturbed study areas demonstrated that filters decayed slowly in comparison with grass litter at the grassland site, filters decayed at the same rate as fir needles at the subalpine site, while filters placed in the undisturbed oil sands plot decomposed more rapidly than jack pine needles. Therefore, for undisturbed systems it is difficult to generalize about the relationship between the decomposition of filters and of natural litter, since the relationship appears to be site specific and is probably dependent on the litter type characteristics, climate, and the speed at which decomposing substrates are incorporated into the soil system.

Ross et al. (1978), investigating the decomposition of cellulose squares and tussock grass litter in New Zealand, made similar observations when he found that the weight losses of cellulose squares placed on the soil surface were generally correlated positively, but not significantly with total weight loss of tussock grass litter and its cellulose component. These authors also noted that variability amongst replicates of the cellulose squares was high and that microenvironment was important in determining decomposition rates. Filter paper weight loss in the present study was also highly variable, particularly in the disturbed sites. A higher degree of variability amongst weight losses of filter paper versus weight losses of native herbage was also measured by Ratliff (1980) in meadow sites in California. Lack of an adequate supply of essential nutrients in the filter substrates could possibly explain some of the variation, since microbial utilization of the cellulose would be somewhat dependent on the nutrient status of the soil under or surrounding the filter. Park (1976) emphasized the importance of mineral nutrients (particularly N) as one of the variables affecting the rate of decay of such substrates as cellulose. Natural plant litter would have a more balanced nutritional status than pure substrates such as cellulose, making the litter more amenable as a food resource for the soil microbes.

There have been few studies to determine the decomposition potential of soils disturbed by surface mining. Lawrey (1977) investigated the relative decomposition potential of strip-mined and undisturbed areas in eastern Ohio. His results showed that decomposition of Pinus, Robinia and Acer leaf litter was initially retarded in soils affected by mining, but after six months there were no significant differences in weight loss of litter between mined and unmined areas. Decay times for litter placed on strip mines in Missouri were reported to be two to 10 times less than those for litter placed on vegetated soils (Carrel et al., 1979). For reasons discussed in the previous paragraph, the filter paper decay data collected for minespoils in this study are difficult to compare with the decomposition rates of natural litter measured by other investigators. Although filters degraded more rapidly over the long term in the mined areas at both the grassland and subalpine sites, it would be dangerous to assume that similar results would have been obtained if natural litter had been tested. Due to the high degree of variation in filter paper weight loss and the lack of relationship between filter decay and natural litter decay, it is suggested that in the future a litter rather than pure cellulose substrate be utilized to study the effects of disturbance such as mining on the general decomposition process. Grass litter produced under standard, light, moisture and nutrient conditions in a greenhouse may be a reasonable alternative.

4.1.7.2 Native plant residues (leaf litter and branchwood). It was considered important to determine the rate of disappearance of native litter and wood in the undisturbed sites, since this type of data would provide a useful baseline if and when the adjacent disturbed systems were eventually reclaimed to a level of productivity similar to that in the undisturbed ecosystems. Decay of native substrates were not followed in the minespoil, since revegetation practices have, to date, emphasized the use of fertilizers and fast growing grass and herb species, resulting in the production of litter whose chemical quality is markedly different from that found in the undisturbed sites. Aside from the abiotic factors (i.e. temperature and moisture) chemical quality of the litter is the main variable influencing rates of decomposition (Bunnell et al., 1977a, b).

The percent dry weight remaining of wheatgrass litter decreased linearly over the first 24 mo of decomposition and then levelled off. This pattern of decay can be explained by the initial rapid loss of the easily degraded constituents of the grass such as soluble sugars, polysaccharides and cellulose followed by the much slower decay of the more recalcitrant components such as lignin. Swift et al. (1979) reviewed a number of studies which demonstrated that as the decomposition of various litter types progressed the decay resistant hemicellulose and lignin fractions tended to accumulate resulting in reduced decomposition.

The weight loss of the grass over the first 12 mo (39%) of this study was very similar to that recorded by Abouguendia and Whitman (1979) for wheatgrass placed in a mixed grass prairie in North Dakota. After 392 days of decomposition, they recorded a weight loss of 39.7%. Much of this weight loss can probably be attributed to a combination of leaching losses and the rapid microbial utilization of the water soluble constituents of the substrate. An estimate of the water soluble component of the Agropyron leaf litter was obtained from a leaching study performed under laboratory conditions. Weight losses ranged from 16.2% after 1 day of leaching to 30.8% after 10 da leaching. Therefore, the weight loss over the first year can be largely accounted for by the disappearance (through leaching or rapid microbial utilization) of the water soluble fraction of the substrate. It is doubtful that leaching would play a major role in the loss of the water soluble component, since the study area receives very little precipitation. In fact, decay of native substrates is probably severely limited by the lack of moisture characteristic of the semi-arid mixed grass prairie.

Turnover time of the mixed grasses was calculated by Abouguendia and Whitman to be 3 yr using data collected by the litter bag technique. Decay rates measured in this study suggest that the turnover time of Agropyron leaf litter is much longer. Enclosing the litter in a mesh bag may have reduced the rate at which it was incorporated into the soil once fragmentation of the leaves had occurred. It is highly likely that the presence of the mesh bag may have reduced decomposition in the same manner as that described for the filter paper.

The rate of disappearance of wood in mixed grass prairie sites has not received any attention, mainly because woody substrates form an insignificant proportion of the aboveground biomass. However, sagebrush occurs regularly on the grassland study site, and appeared to be particularly common where grazing pressure was high. Since the stems of sagebrush tend to be very woody, it was decided that a decay study of these stems would provide some indication of the potential of the grassland system to degrade a woody substrate. Weight loss from the stems was extremely slow with 88% of the stems still remaining after almost 4 yr of decomposition. Compared with the grass, the water soluble component formed a minor portion (2.6-6%) of the total substrate weight, hence the initial rapid decay observed for the grass litter would not be expected during the decomposition of the woody substrate. The faster decomposition of grass litter compared with the woody sagebrush stems can also be explained by the generally higher C/N ratio and lignin content of wood. Also, sagebrush synthesizes aromatic compounds which may behave as toxic inhibitors of microbial activity, thereby reducing decomposition. Swift et al. (1979) have termed compounds such as these, "modifiers", because they affect microbial activity by different mechanisms than those observed for C or nutrient sources. Other factors which would contribute to the decay resistance characteristic of wood, includes the physical nature of wood which because of its density is not conducive to rapid microbial growth and the lack of invertebrate activity. The ability of invertebrates to comminute a resource and disperse microbial propagules could stimulate microbial growth; thereby accelerating decay rates.

The degradation of fir needles in the subalpine spruce-fir forest, did not proceed at a very rapid rate with the bulk of the needles (67%) still remaining after 4 yr of decomposition. Fogel and Cromack (1976) studied the decay of the similar Douglas-fir needle litter at sites in Oregon and measured approximately 65% remaining after 2 yr in the field. The faster decomposition of Douglas-fir needles compared with the decay rates of the subalpine fir needles in this study are probably the result of climatic differences with more amenable moisture and temperature conditions accelerating the disappearance of fir needles in the Oregon study. Aside from the inhibitory effects the cool temperatures characteristic of the subalpine site can have on the decay process, the other factors which would possibly account for the relatively slow disappearance of the subalpine fir needles include:

i) a possibly higher C/N ratio and lignin content in fir needles than that in herb or grass litter,

ii) a lower proportion of easily degraded water soluble constituents. Leaching losses from fir needles ranged from 3-7% after 1 da submersion in water to 17.6% after 10 da submersion,

iii) the presence of a wax layer on the needles which can restrict entry of water and decomposers into the needle interior,

iv) the presence of a high level of polyphenols in the needles.

As was mentioned previously for the filter paper placed in the undisturbed spruce-fir site, the incorporation of the decaying needles into the litter/soil system was very slow and appeared to occur only when the feather moss had overgrown the litter bag. Since measurement of the rate of decay of needle litter is still in progress, it will be interesting to see if decay accelerates once the bagged needles become part of the moss/litter layer.

The decomposition of subalpine fir branch wood was very much slower than that of the fir needles - an observation also made by Fogel and Cromack (1977) for Douglas-fir branch wood. As discussed for the sagebrush stem decay data, the differences observed between the disappearance of litter and wood, are mainly a product of differences in resource quality, particularly in lignin content. Fogel and Cromack (1977) concluded from their study that annual weight loss of the various litter types was more influenced by lignin content than C/N ratio. They measured a lignin content of 21.8% in the needles vs. 43.4% in the branch wood which would explain why the branch wood in their study was more resistant to decay.

Weight loss of bagged jack pine needles was highly variable amongst the five sampling times. This variability was mainly a result of the inefficient removal of sand from the surfaces of the needles. Since the needles were not ashed, weight gains such as that recorded between 24 and 33 mo resulted. The adhesion of sand to the litter would also cause an underestimation of the decay rate of the needles. Litter bags containing the needles were placed under the lichen (Cladina mitis) mat since the lichen would have restricted the movement of the bag into the sand beneath. As a comparison, the decomposition of jack pine needle fascicles strung on nylon thread and placed within the lichen mat was also investigated. Over the first 24 mo of decay the strung needles decomposed more slowly than those in bags below the lichen, mainly because the strung needles were more subject to extremes in temperature and moisture. Weight loss of the strung and bagged pine needle litter was approximately 16% and 22% respectively after 12 mo compared with 30% obtained for jack pine needles by Maclean and Wein (1978) in a forest stand in New Brunswick. Higher precipitation experienced by the New Brunswick study areas would account for the faster disappearance of jack pine needles than that measured at the oil sands site. Also, the absence of a lichen mat in the New Brunswick jack pine stands could speed up the rate at which incoming litter is incorporated into the litter soil system - a factor which may enhance decay rates. The relatively slow disappearance of jack pine needles in comparison with that of wheatgrass at the grassland site is similar to that observed for fir needles at the subalpine site. It is possible that the same resource quality factors reducing decay of the fir needles also reduce decay of the jack pine needles.

Maclean and Wein (1978) reported weight losses of 6-10% after 12 mo and 7-15% after 24 mo for jack pine branches placed in various tree stands in New Brunswick. Similar data were obtained for jack pine branchwood in this study with 8% wt loss after 12 mo and 12% after 24 mo. Interestingly the rate of decay of the jack pine branch wood over the first 24 mo of the study was approximately the same as that measured for the jack pine needles. One explanation for this observation is that moisture is the predominant variable controlling decomposition at the oil sands study area. Until moisture conditions are more conducive to microbial growth (i.e. when the needles or branches are well embedded in the lichen mat or have worked their way into the soil beneath the lichen), resource quality plays a very minor role in the decay process. More research is required to establish the importance of the moisture variable relative to the temperature and resource quality variables in the decomposition process occurring in jack pine stands at the oil sands site.

4.1.8 Organic matter accumulation and development of microbial activity and biomass. Since microorganisms require C more than any other nutrient for their growth and tissue synthesis, one would expect that as revegetation of a minespoil progresses and organic matter accumulates, microbial activity and biomass C would increase accordingly. But, with the exception of Stroo and Jencks (1982), very few studies have been conducted on the development of microbial activity in minespoils as soil genesis progresses. Stroo and Jencks observed that microbial activity in minespoils in W. Virginia increased as the age of the revegetated minespoil increased and concluded that recovery was related to organic matter and N levels. In fact, respiratory activity was highly correlated with biologically available C and mineralizable N. Also, microbial respiration in unamended sites revegetated for 20 yr approached that in the native soils. In the present study, microbial respiration, biomass C and organic matter levels in the subalpine minesite all demonstrated increasing trends as the age of the minespoil increased. It is worthwhile noting that CO_2 evolution, biomass C and organic matter levels were not significantly different in the undisturbed mineral soil and stockpiled regolith which infers that it is the removal of the vegetation and organic matter and not the physical disturbance of the soil which controls the microbial activity in the soil after mining.

Plant growth in a minespoil results in the accumulation of organic matter as the products of primary productivity (shoots and roots and root exudates) die and are decomposed by the soil microbial component. The rate of organic matter accumulation will be determined largely by plant species, plant productivity and the rate of decay of incoming plant litter. The significant increase in organic matter between the 6 and 7 yr old sites is difficult to explain. A detailed history of each site was not available so it is possible that different fertilizer regimes and the seeding of different plant mixtures may have influenced the rates of organic matter accumulation in the 6 and 7 yr old sites. Although microbial activity and biomass C in the 7 yr old site was significantly greater than that measured in other other sites, it was still substantially less than that in the undisturbed organic mat. These observations suggest that organic matter accumulation on the subalpine minespoil is a very slow process, a conclusion also drawn by Wilson (1965) for vegetated spoils in West Virginia.

Like Stroo and Jencks (1982), a highly significant relationship was detected between microbial biomass C and percent organic matter. A regression analysis performed on biomass C and organic matter measurements from the disturbed soils only demonstrated a strong relationship between the two variables but not as strong as that detected when measurements from the undisturbed soils were included. It is considered necessary to analyze more soil samples (particularly those with organic matter ranging from 40 to 80%) before these regressions can be utilized as predictive models.

4.2. TANK STUDY

4.2.1 Rationale for the Tank Study

The evidence provided by the field study demonstrated conclusively that mining disturbance at all three study sites had an adverse effect on soil microbial activity, particularly when the surface disturbed and undisturbed soil layers were compared. Associated with the decrease in microbial activity in the minespoils was a reduction in bacterial, actinomycete numbers (with the exception of the grasslands site) and hyphal lengths and major shifts in the bacterial and fungal community structures. The loss of much of the soil organic matter during the mining process, was postulated as being the main factor affecting soil microbial activity and community dynamics since it is the organic matter which serves as the energy source for microbial growth. Also root growth tends to be concentrated in the organic horizon of the soil profile (grassland and subalpine sites in particular), hence the destruction of this horizon would eliminate the microbial activity associated with the roots (rhizoplane, rhizosphere, mycorrhizae). Therefore, if the re-establishment of microbial activity is to be considered an essential step in the successful reclamation of a minespoil, the presence of organic matter (barring the spoil has no extreme chemical/physical problems) would be an absolute necessity. The organic matter levels in a minespoil can be raised by establishing a self-maintaining vegetative cover (which would ultimately result in the accumulation of organic matter through the decomposition of plant residues) or by amelioration with an organic amendment and subsequent revegetation.

In the present study, it was hypothesized that the incorporation of an organic amendment into a minespoil would not only serve to re-establish soil microbial activity, but would ultimately result in the more rapid establishment of a stable vegetative cover. Consequently, a large quantity of minespoil from each of the three study areas was transported to Calgary, where it was amended, planted and monitored regularly for such parameters as plant success and growth, development of microbial activity and biomass C and decomposition potential. The amendments which were tested on the grassland spoil were gypsum, sewage sludge and topsoil while those applied to the subalpine and oil sands spoil included fertilizer, sewage sludge and feather moss peat. Details of the tank design, application rates of the amendments and the plant species tested have been outlined in Visser et al. (1984).

4.2.2 Microbial Characteristics of the Individual Amendments

Of the five amendments, the anaerobically digested sewage sludge exhibited the highest microbial activity and ATP content explained mainly by the nutrient-rich composition of the sludge. The lack of biological activity in the fertilizer and gypsum was to be expected since these amendments tend to be low in moisture, highly acidic with very little biologically available carbon and potentially toxic levels of some nutrients (e.g. ammonium N in the fertilizer). Microbial respiration, numbers of bacteria and hyphal lengths in the peat and topsoil were substantially greater than that measured in the fertilizer and gypsum, but not as high as that measured in the sewage sludge presumably because the nutrient status of the sludge was better than that of the peat and topsoil (see Table 6a, Visser et al., 1984). Only the peat and topsoil harbored substantial numbers of actinomycetes. The microbial and nutrient composition of the various amendments would infer that microbial activity, bacterial numbers and hyphal lengths in the various minespoils would over the short term be most stimulated by the addition of sewage sludge and least by the gypsum and fertilizer. However, this was not the case in this study since sewage sludge was added to the minespoils at a very low rate (46 mT ha^{-1}) resulting in a dilution of the microbial inoculum and nutrients within the soil matrix. In contrast, topsoil and peat were applied as 15 cm layers resulting in the application of a heavy dose of microbial inoculum to the minespoils in the case of these two amendments.

It is worthwhile noting at this point, that the sewage sludge was mixed into the soil after it had been allowed to dry on the soil surface. The nature of the dried sludge caused great difficulty in establishing a homogeneous sludge soil mixture. Rather the sludge was dispersed through the soil as clumps of hard carbonaceous material which represented nutrient-rich islands of intense microbial activity.

4.2.3 Microbial Development in Amended Prairie Grasslands Minespoil

4.2.3.1 Microbial numbers. The application of sewage sludge to the grasslands spoil increased the number of bacteria in the spoil surface to a greater degree than the gypsum and topsoil treatments. It is difficult to determine whether this increase was a result of the introduction of bacteria with the sewage sludge or the stimulation of reproduction by the indigenous soil bacteria resulting from the highly available nutrients present in the sludge. The stimulatory effect of the sewage sludge on the bacterial population was relatively long-lived since bacterial numbers in the sludge treated spoil were still significantly higher than in the other treatments at 15 mo after amendation. The growth of fall rye in the sludge treated plots over the first 15 mo of the study may also have contributed to the stimulation of bacterial growth particularly in the rhizosphere. Frequez and Lindemann (1982) have reported a significant increase in the number of aerobic heterotrophic bacteria in the rhizosphere of Atriplex canescens when grown in sodic coal spoil amended with sewage sludge.

The treatment of the spoil with gypsum to relieve the sodicity problem characteristic of this minespoil did not affect the numbers of bacteria, but did appear to reduce the number of actinomycetes, particularly those in the subsurface soil. The application of the highly acidic gypsum (pH 3.0) may have had an adverse effect on the actinomycetes since the streptomycetes, which can form a large proportion of the actinomycete population, are sometimes highly sensitive to acid (Kuster, 1967). Streptomyces spp. have been shown to be salt tolerant, preferring alkaline, dry, loamy, organic soil (Kuster, 1967) which explains their abundance in the grassland topsoil. The general increase in actinomycete numbers in the 5-15 cm soil over the first 15 mo of the study can be partially explained by the development of a rhizosphere effect around the fall rye roots.

4.2.3.2 Shortcomings of ATP measurements. As was the case in the field studies, ATP determinations of the variously amended grassland minespoil was so variable that no definitive conclusions could be drawn from the data. Therefore, this technique was abandoned for any further monitoring on the effects of the amendments and plant growth on the development of microbial activity in the minespoil. Schafer et al. (1979) reported the use of ATP measurements to investigate the microbiological development which had taken place in 1 to 50 yr old stripmine spoils in Montana. Unfortunately, their data did not include any measure of the variation they encountered, making it difficult to interpret their results.

4.2.3.3 Organic matter accumulation. As mentioned previously, a productive vegetative cover on a minespoil provides the energy required by microorganisms for their growth and tissue synthesis. The rate at which the microbial component utilizes its food resource (i.e. plant litter, dead roots etc.) will eventually determine the rate at which stable organic matter accumulates. Shoot production by fall rye in the grassland minespoil was highest in the sewage sludge and topsoil treatments; therefore litter input was stimulated by these two amendments. Root production and consequently dead root input was, however, significantly greater in the topsoil treated spoil than in the sludge treatment. The high N level in the sewage sludge may have resulted in a reduction of the root system relative to the shoots (Russell, 1977). Unfortunately, primary production by fall rye ceased after the second growing season - a factor not anticipated when this study was initiated. Whether a plant species chosen to revegetate a minespoil is short or long-lived is an important factor to consider if one of the goals of reclamation is the accretion of organic matter on the minespoil. Although significant quantities of fall rye litter and roots were deposited in the sewage sludge and topsoil-amended plots, plant growth did not influence the organic matter content in these two treatments. Organic matter accumulation is probably so slow, and the technique used to estimate organic matter (loss on ignition) so coarse that litter input over many years would be necessary before an increase in soil organic matter content could be measured. Schafer et al. (1979) estimated that 50 years would be required before organic carbon accumulation in the top 1 cm of minespoils in Montana reached equilibrium, while 200 to 400 years would be required for such equilibrium in the minespoil below 10 cm. These present data also suggest that organic matter accumulation on minespoils is a relatively slow process.

4.2.3.4 Microbial activity and biomass. Immediately after amendation both microbial activity and microbial biomass C exhibited a decreasing trend from the topsoil treatment down to the untreated minespoil. Topsoil was the most effective in improving the microbial status of the minespoil, since this amendment was characterized by high levels and a wide variety of microorganisms and was applied at a fairly heavy rate in comparison with the other two amendments. Sewage sludge with its high microbial inoculum load and nutrient rich composition was also highly effective in raising the microbial activity and biomass in the minespoil. Eiland (1981) and Macgregor and Naylor (1982) reported similar observations when they applied sewage sludge to cropland. The treatment effects occurred mainly in the top 5 cm of the minespoil, which would be expected since this is where much of the amendment was concentrated after its application.

With the exception of Wilson (1965) who reported an increase in microbial respiration of a coal minespoil after the addition of household refuse and Stroo and Jencks (1982) who used O_2 uptake to characterize minesoils varying in age and vegetation, very few studies have used soil respiration (i.e. O_2 or $CO_2\uparrow$) as a means to assess the ability of various amendments and planting regimes to improve the microbial activity in a minespoil. This is surprising since the measurement of soil respiration is one of the most rapid and direct methods for obtaining an insight into the effects of various parameters on soil microbial activity.

The CO_2 efflux from the surface of the variously amended spoil planted with fall rye peaked at 15 mo after planting. At this point, shoot production by fall rye had also attained its peak which suggests that microbial activity in all the treatments had been stimulated by plant growth. The readily available C provided to the soil microorganisms through sloughed root material and root exudates is believed to have been the main factor stimulating microbial activity during the second growing season. It is widely recognized that plant roots release a wide variety of substances (amino acids and carbohydrates) when they are actively growing (see Russell, 1977). Little is known about the quantity of photosynthate released from plant roots through exudation and sloughing. Barber and Martin (1976) attempted to quantify root exudation and estimated that 12-18% of the photosynthetically fixed carbon of wheat and cereal plants was released by the roots. In the nutrient-poor conditions characteristic of minespoils, this amount of C could represent a major food resource for the soil microflora. The importance of root exudates as a source of C for stimulating microbial growth and activity in minespoils has been confirmed by Wilson (1965) and Fresquez and Lindemann (1982).

Plant growth did not appear to influence microbial activity in the subsurface spoil which infers that much of the root activity during the second growing season occurred in the 0-5 cm deep soil. After the third growing season, microbial activity in the top 5 cm had decreased to levels similar to those measured 0.5 mo after amendation. Interestingly, primary production by fall rye dropped sharply during the third growing season which suggests that soil respiratory activity may indeed be linked to plant growth. In the early stages of reclamation, this link may be solely dependent on levels of root activity since litter accumulation has not yet taken place. In terms of microbial activity measurements, the variously amended grassland spoil regressed to its pre-planting stage mainly because fall rye is a short lived grass which ceased to grow after two growing seasons. Stroo and Jencks (1982) stressed the importance of vegetation to the development of microbial activity in minespoils particularly in relation to the release of root exudates. As in this study, these authors observed that when the vegetation on a minespoil failed (even after 10 years), microbial activity reverted back to levels similar to those prior to revegetation.

4.2.3.5 Microbial biomass C. As was the case for microbial activity, microbial biomass C in the 0-5 cm deep, amended minespoil also increased significantly over the first 15 mo of the study. It is assumed that the same factors were associated with the biomass stimulation as with the respiratory activity stimulation since these two parameters are often closely related, particularly when a highly available C source is present in the soil. Unlike microbial respiration, microbial biomass C did not decrease between 15 and 27 mo after amendation; instead it appeared to level out over this time period. Although microbial respiration appeared to be related to plant growth, this was not true for the microbial biomass. A possible explanation for this is that microbial biomass measurements are based on soil respiratory activity after the addition of a readily metabolizable substrate (i.e. glucose). Therefore, microbial biomass C is measured when soil nutrient conditions (i.e. C) are not limiting to the microbial populations, whereas microbial respiratory activity ($CO_2\uparrow$ or $O_2\downarrow$) is measured under varying C conditions. As mentioned previously, the microbial activity responsible for the oxidation of a particular resource is controlled by three main factors: moisture, temperature and the chemical composition of the resource (i.e. substrate quality). As a result, microbial respiration measured under constant temperature and moisture conditions, will vary with the amount of soil C readily available to the microorganisms. Peak CO_2 evolution after the second growing season can be explained by the elevation in the quantity of C available to the soil microflora from root exudates or from the amendments. This source of C was negligible during the third growing season, hence microbial activity decreased. However, the microbial biomass responsible for this activity did not decrease; rather, its activity was probably limited by a lack of C. The addition of C in the form of glucose allowed the C-limited biomass to express its potential under non-limiting conditions. It appears, therefore, that the growth and turnover of microbial tissue (as evidenced by CO_2 efflux) was greater during the second growing season because of the increase in highly available (root exudate) C. The loss of this C during the third growing season resulted in less microbial growth and turnover, but there was enough C available for the biomass to maintain itself although not at the level of metabolic activity observed after the second growing season. These results suggest that the rate of microbial growth and turnover may be closely linked to plant productivity in the variously amended minespoils, whereas the microbial biomass maintains itself for some time after the cessation of plant growth. Undoubtedly, the microbial biomass would also be adversely affected if no plant growth occurred for an extended time period (i.e. when the C required to maintain the microbial biomass was no longer available). In the subsurface spoil microbial biomass, like microbial activity, changed very little over the course of the study.

Correlation coefficients calculated between grass root and litter weights and microbial activity and biomass after the third growing season implied that a reasonably close relationship existed between dead plant litter input (particularly roots) and soil microbial biomass. This relationship may be true but caution should be exercised when searching for relationships using correlation coefficients. For example, the high correlation observed between grass root weight and microbial activity and biomass may be an indirect relationship between amendment nutrient quality and microbial activity. Since both the highest root weight and microbial activity and biomass occurred in the topsoil treated spoil, followed by sewage sludge, gypsum and untreated spoil, it is possible that amendation rather than plant growth (which had ceased during the third growing season) determined levels of microbial activity.

4.2.3.6 Decomposition process. The decomposition potential of the various treatments (as determined by the decay of filter paper) exhibited different patterns of weight loss depending on vegetation type. Over the short term (1 yr) filters placed in both the fall rye and rambler alfalfa planted plots decayed most rapidly in the topsoil treatment followed by the sewage sludge, gypsum and control treatments. However, after two years in the field, the decomposition of filters in the fall rye plots demonstrated no amendment effects, whereas the filter papers placed in the alfalfa plots followed the same pattern of decay observed during the first 12 mo of the study. These observations suggest that it was vegetation type rather than the amendment which controlled the rate of decay of the filters. If the filter decay results are viewed in conjunction with shoot production data (see Visser et al., 1982), one observes that short term decomposition in the fall rye plots was fastest in the treatments with the highest shoot production (topsoil and sewage sludge) and slowest in the treatment with poor plant growth (gypsum and control). As mentioned previously for the field sites, Ross et al. (1978) reported that decay of pure cellulose squares placed on the soil surface in tussock grasslands in New Zealand was controlled mainly by micro-environment. It is believed that variations in microclimate caused by differences in foliage density produced on the various treatments was also the main factor determining the decay of cellulose filters in this study. During the third growing season, growth of fall rye ceased, hence the microclimatic effects would presumably have been less pronounced. Although filters placed in the alfalfa plots demonstrated similar treatment effects as those placed in the fall rye plots, the differences in weight loss (particularly over the first 12 mo) between filters in the plots with greatest shoot production and those in the plots with lowest shoot production were not as pronounced as observed for the fall rye treatments. This can be explained by the longer period of time required by alfalfa to establish itself in the topsoil treatment, and the poor germination and establishment of this plant species in the other treatments. As a result, density of the alfalfa plants was lower than that observed for fall rye and therefore the effects of microclimate on the decay of filters in the alfalfa plots was less evident and more variable. Rambler alfalfa, however, is a longer-lived species than fall rye, hence the amelioration of microclimate near the soil surface in areas planted with this species would be expected to be longer term and improve as primary production increased. This may have occurred in the topsoil treated plots over the 12-24 mo term of the study, since filters decayed rapidly in this treatment over this time period. It appears, therefore, that amendation of a minespoil may over the short term indirectly affect the potential of a spoil to degrade a pure substrate such as cellulose, by determining the germination, establishment and primary productivity (all factors which will affect plant density and microenvironmental variables) of the vegetation planted in the spoil. More research is required to discriminate between the amendation effects and the effects of plant density on the decomposition process.

The importance of vegetative cover to the decay of cellulose was substantiated when the degradation of filter paper over the short term (12 mo) was again tested 28 mo after amendation and planting fall rye and alfalfa. A comparison of the decomposition rates of filters placed in the fall rye plots 4 mo after planting and again 28 mo after planting showed that the cellulose decay potential of the two treatments which had exhibited the greatest shoot production (i.e. topsoil and sewage sludge) decreased over the 40 mo term of the study. The decay of filters placed in the control and gypsum treated plots where shoot production had been low, was much less influenced by time. These data infer that during the second growing season (when fall rye shoot production peaked) filter degradation was significantly accelerated by a dense vegetative cover. The chemical and biological properties of the amendments appeared to have very little direct effect on the decay rates, because when growth of fall rye ceased no differences were detected in decay rates amongst the four treatments. It is, of course, highly probable that as moisture and temperature conditions at the soil surface improve with increasing plant cover and dead litter accumulation, the nutrient quality of the litter and amended minespoil would be of increasing importance in regulating cellulose decay. Obviously the link between the vegetation in a minespoil and its microbial activity and cellulose decay potential is a crucial one. When the growth of a plant species fails (as occurred in this study with fall rye), there is a danger of a minespoil regressing to its pre-planting state which it did in the topsoil treated plots.

Unlike the cellulose decomposition observed in the fall rye plots, cellulose decay potential in the rambler alfalfa plots increased significantly with time (particularly in those plots treated with sewage sludge and topsoil). As mentioned previously rambler alfalfa is a long-lived perennial, whose primary production increased during the term of the study. A combination of microclimatic effects caused by the vegetative cover, dead litter accumulation at the soil surface and the nutrient quality of the litter and amended minespoil surrounding the litter bag would have been the main factors governing cellulose decay in the alfalfa plots.

Wood dowel decomposition in the tank plots was as slow as that recorded in the field sites - an observation explained primarily by the decay-resistant (high lignin) quality of the wood. The very slow decay exhibited by this particular substrate makes it a rather useless material for comparing the decay potential of soils in various stages of reclamation, unless measurements are made over a very long term.

4.2.3.7 N_2 fixation by rambler alfalfa. Since minespoils are accepted as being notoriously low in plant available N, it has been recommended (Skeffington and Bradshaw, 1980) that legumes be planted to accelerate the rate of nitrogen accumulation. In order to ensure that these legumes become well established with well nodulated roots capable of N_2 fixation, it is important that the effects of various amendments on legume growth be recognized. The amendments used to ameliorate the grasslands spoil (i.e. sewage sludge, gypsum and topsoil) did not appear to inhibit the formation of N_2 fixing nodules during the first growing season. Unfortunately the data were highly variable at all sampling times which makes it difficult to draw any definitive conclusions on the effects of the various amendments on N_2 fixation potentials. The heterogeneous distribution of the sewage sludge and gypsum, and the difficulty in extracting complete root systems from the various treatments undoubtedly contributed to the variation. Nevertheless the results infer that even in the untreated spoil the nutrients required for successful infection, nodulation (mainly calcium) and N_2 fixation (phosphorus, sulphur and micronutrients) by Rhizobium were available in sufficient quantities. Although sewage sludge did not have a significant inhibitory effect on N_2 fixation during the first growing season, caution should be exercised when applying this amendment to legume planted minespoils. Sewage sludge contains high levels of ammonia and other forms of nitrogen which have been shown to inhibit both nodule formation and nitrogen fixation (Smittle, 1979). N_2 fixation potential of alfalfa in the sewage sludge treated minespoil increased substantially from the first to the second growing season, so if the sewage sludge contained any inhibitory substances when first applied, detoxification (perhaps through oxidation of ammonia and other N transformations) had occurred by the end of the second growing season.

Nodule weight and N_2 fixation appeared to be highest after the second growing season, particularly in the sludge and gypsum treatments. Perhaps nutrients released by these two amendments at this time stimulated plant growth, thereby causing an indirect stimulation of N_2 fixation. The difference in nodule morphology between plants from the topsoil treatment and the other three treatments is interesting. The small size of nodules on plants in the topsoil treatment may be attributed to such factors as deficiencies of molybdenum, phosphorus or sulphur (see Munns, 1977) or high plant density (Graham and Rosas, 1978).

Skeffington and Bradshaw (1980) determined that the role of the free-living bacteria in accumulating N in derelict land was negligible when compared with N input from N_2 fixing leguminous plants. This study supports their conclusions since N_2 fixation potential of the topsoil and untreated spoil was .15 and 0 nmoles 100 g^{-1} dry soil hr^{-1} respectively compared with 93.7 and 149.5 nmoles g^{-1} dry root hr^{-1} for alfalfa grown in the topsoil and untreated spoil respectively for two growing seasons.

The extreme variability recorded in this study is problematical. Further research is required to improve the precision of the acetylene reduction technique particularly when applied to plants grown in widely contrasting soils. Trinick et al. (1976) reported that field grown plants of Lupinus luteus were highly variable in their acetylene reducing ability and that ten groups of five plants were required to reduce the coefficient of variation to 0.1. Hence, considering the highly variable nutrient conditions, particularly in the sewage sludge and gypsum treated plots, it would appear that an enormous number of plants would be required from each treatment to obtain dependable, consistent results. It is possible that if N_2 fixation potential of a legume crop was measured in the field over fairly large areas, the variation would be reduced considerably.

4.2.4 Subalpine Spoil

4.2.4.1 ATP, microbial numbers and fungal mycelial lengths. Microbial population estimates as determined by ATP bacterial and actinomycete counts and fungal hyphal length measurements demonstrated that shortly after amendation, bacteria were most numerous in the sewage sludge and peat treatments, while the greatest number of actinomycetes and amounts of fungal hyphae occurred in the peat treatment. ATP levels were generally higher in the sewage sludge and peat treatments, but the extreme variability in the ATP measurements made it difficult to analyze the data. As a result ATP estimates were not considered an accurate means for monitoring microbial development in the variously amended plots, and were therefore terminated. It is not surprising that the minespoil treated with sewage sludge and peat exhibited the highest levels of microbes since these two amendments introduced not only large numbers of micro-organisms, but also C and essential nutrients, such as N and P, required by the microbes for growth and maintenance. Fertilizer contained very few organisms prior to its application, and lacked the C necessary for stimulating the growth of the minespoil microflora. As in the grassland spoil, the immediate effects of the amendments on microbial numbers occurred mainly in the upper 5 cm of the treated spoil where the amendments tended to be concentrated.

Plant growth for two growing seasons did not significantly alter bacterial numbers in the 0-5 cm deep amended minespoil; however in the deeper, subsurface spoil, both bacterial and actinomycete numbers in all the treatments had increased significantly after growing slender wheatgrass for 15 mo. This increase may have been stimulated by root exudates since slender wheatgrass shoot production and presumably root production peaked during the second growing season. The potential importance of root productivity and exudation to microbial growth has been discussed previously for the grassland spoil. The lack of a response by the fungal hyphae to plant growth suggests that the bacteria and actinomycetes may respond to root exudates to a greater degree than the saprophytic soil fungi. The significant reduction in actinomycete numbers in the 0-5 cm zone of peat treated minespoil over the first 15 mo of the study is difficult to explain. Perhaps the reduction in levels of N and P in the peat (see Visser et al., 1984) adversely affected actinomycete populations. Also, the peat had been freshly excavated prior to its application to the minespoil. Changes in some of the physical and chemical qualities of the peat as a result of exposure may have caused a reduction in actinomycete numbers.

4.2.4.2 Microbial respiration and biomass in unplanted minespoil. The higher microbial numbers measured in the top 5 cm of the peat and sewage sludge treated minespoil supported the microbial activity determinations which were also observed to be greater in the peat and sewage sludge amended spoil than in the fertilized and unamended spoil. Soil respiration in the subsurface soil was minimally affected by treatment until 15 mo after amendation when CO_2 efflux at both depths in all treatments rose significantly. This increase was most obvious in the sewage sludge and peat amended minespoil and may have been partially due to a release of nutrients from the amendments which in turn stimulated microbial activity. After this peak in microbial activity in the amended, but unvegetated minespoil, respiration in the surface soil returned to what it was shortly after amendment application, while CO_2 efflux in the 5-15 cm spoil dropped significantly lower than that recorded at the first sampling. These results suggest that any highly available nutrients, particularly C, present in the amendments had been utilized by the end of the study. However, estimates of C (measured by loss on ignition) in the variously treated unvegetated minespoil over the term of the study implied that on a very crude scale, no alteration of C levels occurred. It can, therefore, be concluded that the major portion of the C introduced with the sewage sludge and peat was of a very stable nature and not susceptible to massive microbial oxidation over the short term.

The pattern of microbial biomass development was very similar to the microbial activity pattern with the exception that biomass C (particularly in the surface soil) did not decrease after 15 mo. Interestingly, this pattern resembled that obtained for the grassland spoil planted with fall rye, suggesting that similar factors controlled microbial activity and biomass in the two spoils. As in the grassland spoil, a reduction in the quantity of highly available C in the subalpine spoil from 15 to 27 mo may have decreased microbial activity (i.e. microbial growth and turnover); however, there was enough C available for the biomass to maintain itself at a reduced level of metabolic activity after the highly available C had disappeared.

4.2.4.3 Microbial respiration and biomass in plots planted with slender wheatgrass or white spruce. Previous investigations have suggested that vegetation is crucial to microbial activity in minespoils (Stroo and Jencks, 1982) and that the species of plant used in the revegetation program may determine the rate of development of microbial activity (Müller, 1973). Since white spruce and slender wheatgrass grow at very different rates and have distinctly different root morphologies, it was decided to follow microbial activity and biomass in the variously amended plots planted with these two plant species. The results were then compared to those obtained from amended, but unvegetated spoil.

Over the term of the study, microbial activity and biomass C estimates in spoil planted with white spruce closely resembled those measured in the unplanted spoil with the exception of the upper 5 cm soil from the peat treatment. Here, microbial respiration and biomass in peat removed from the white spruce plots at 27 mo after planting was somewhat higher than that measured in the unvegetated peat treated spoil. White spruce root weights after three growing seasons were heavier in the 0-5 cm soil in the peat treatment than in the sewage sludge, fertilizer and control treatments. Therefore, it is possible that root activity in the peat stimulated microbial activity to a greater degree than roots in the other amendments. However, it is worth noting that ectomycorrhizal infection of the white spruce roots was extremely high (Danielson et al., 1984) and that microbial stimulation resulting from root exudation may have been influenced by the presence of the mycorrhizal fungus. In the subsurface soil from the sewage sludge treated plots, microbial biomass C increased significantly between 0.5 and 27 mo after amendation, while no changes occurred at this depth in the other treatments. After three growing seasons, there was approximately twice as much root weight in the subsurface soil in the sludge amended plots than the surface soil; therefore, root activity and nutrients released by the decomposing sludge clumps may explain this increase in microbial biomass.

Microbial activity and biomass C at the conclusion of the third growing season, were higher in the plots planted with slender wheatgrass than in the unvegetated spoil and the spoil planted with white spruce. This observation applied mainly to the 0-5 cm deep amended spoil, and was particularly true in the sewage, fertilizer and unamended plots. Peak primary production by slender wheatgrass occurred during the second growing season, resulting in the accumulation of dead litter (leaves and stems) on the spoil surface after this time. Also root weights measured in the grass plots, 27 mo after planting were substantially greater than those measured for white spruce. Sewage sludge amendation was particularly effective in enhancing grass productivity, hence the greatest amount of litter input occurred in this treatment. Root production was highest in the peat amended spoil. Although some of the amendments (sewage sludge and peat) greatly stimulated plant production, loss on ignition measurements in the grass plots did not vary over the term of the study, inferring that this method is not very sensitive for detecting small changes in organic matter, particularly in spoils contaminated with geologic forms of carbon. The soil microorganisms, however, are highly sensitive to the addition of carbon sources such as those found in litter, roots and root exudates and no doubt their growth and activity were greatly enhanced as a result of grass litter and root input during the third and fourth growing seasons. This would have been particularly true in the untreated and fertilized spoil where the supply of highly available carbon was dependent almost entirely on plant growth. These data suggest that the development of microbial activity and biomass is strongly linked with levels of primary productivity mainly because the metabolic activity of the microorganisms is regulated by the availability of an energy source. As a result, it would be expected that the development of microbial activity would be more rapid in a minespoil planted with a fast growing, highly productive grass species than a slow growing tree species, particularly if productivity was enhanced by the addition of a nutrient rich amendment. As in this study, Müller (1973) reported a more active development of microflora in coal mine spoils planted with a grass-legume mixture than in tree vegetated spoils.

4.2.4.4 Comparison of microbial development in amended minespoil planted with grass, spruce or left unplanted. A comparison of microbial development in the unvegetated minespoil and the tree and grass vegetated minespoil demonstrated that over the first three years of this study, the presence of a grass cover was most effective in stimulating the activity and growth of the microorganisms. To increase or maintain the microbial biomass stimulated by the grass over the long term it would be essential that this vegetative cover remain productive. This was, unfortunately, not the case in this study since the growth of slender wheatgrass peaked in the second growing season and collapsed during the third growing season bringing litter and root input (i.e. future organic matter) into the soil system to a standstill. Although the microbial biomass increased and maintained itself during the third growing season (mainly as a result of litter input at this time), there was some evidence that microbial activity was in the process of declining during the fourth growing season (27-39 mo). Further sampling would be required to establish with certainty that the cessation of plant growth caused a reduction in microbial activity. Nevertheless, it appears that the choice of plant species, and the longevity of a plant species once established are important factors to consider when revegetating minespoils since this study suggests that the rate of microbial development is closely associated with the type of vegetative cover. A failure in the vegetative cover could return the soil microbial system back to its pre-planting phase. When using short-lived species such as slender wheatgrass to provide initial erosion control of a minespoil, it may be advantageous to plant longer lived woody shrubs or conifers once the grass cover has failed. The choice of plant species would of course be heavily dependent on the final land use goal.

Microbial respiration and biomass followed the same pattern in both the vegetated and unvegetated minespoil (i.e. they were highest in the peat, followed by sewage sludge, than fertilizer and lastly control). Planting slender wheatgrass seemed to enhance the effects of the amendments more than planting white spruce presumably because of the higher productivity of the grass. It appears, therefore, that levels of plant available nutrients introduced with an amendment determines the degree of primary production (particularly of fast growing species) which in turn regulates the rate of microbial development in a minespoil. There was some evidence that fertilizer, shortly after its application, inhibited microbial activity in the upper 5 cm of the spoil. It is possible that fertilizer inhibition did occur since some investigations have demonstrated a reduction of soil microbial activity in the presence of N fertilization (Kowalenko et al., 1978) while ammonia has been shown to be a volatile inhibitor of fungal spore germination (Pavlica et al., 1977; Setua and Samaddar, 1980). Although the 0-5 cm deep soil in the slender wheatgrass plots was the "action zone" in terms of microbial development, microbial biomass measurements in the deeper 5-15 cm also demonstrated a slow but progressive increase over the first three years of the study. This increase is attributed mainly to the profuse growth of the fibrous grass roots at this depth since the microorganisms in the unamended spoil as well as in the treated spoil exhibited similar patterns of biomass development.

The microbial biomass data collected for the variously amended subalpine minespoil suggested that plant productivity and microbial development were closely related; however, correlation coefficients calculated for data collected 39 mo after planting revealed a poor relationship between grass litter/root weights and microbial activity/biomass. The lack of a relationship may be a result of the interactive influence of the chemical composition of the various amendments and plant growth on microbial activity and biomass. Plant growth and litter input in a carbon limited situation may stimulate microbial activity to a greater degree than plant growth in a minespoil where other forms of carbon have been introduced via highly organic amendments. The relationship between plant and microbial productivity in soils containing different amounts and qualities of organic carbon warrants further study. Microbial activity and biomass, however, were highly correlated with loss on ignition. The wide gap between the organic matter levels in the untreated, fertilized and sludge amended spoil vs. the peat treated spoil partially explains this strong relationship, hence these data should be treated cautiously until soils with a wide variety of organic matter levels have been tested.

4.2.4.5 Decomposition of standard substrates (filter paper and wood dowel). The potential of the minespoil to degrade cellulosic filter paper was most enhanced by amendation with sewage sludge. Unlike the different decay rates of filters placed in the grassland minespoil planted with fall rye and alfalfa, filter decay in the subalpine spoil followed the same pattern regardless of vegetation type (i.e. the decomposition rate of the filters in each of the four treatments was approximately the same whether planted with grass or white spruce). The lower shoot production by slender wheatgrass compared with fall rye, may not have ameliorated the microclimate at the soil surface in the subalpine plots to the same extent as fall rye did in the grassland minespoil, thereby decreasing the effects of microclimate on decay. The cellulose degradation in the sewage sludge treatment appears to have been stimulated mainly by the introduction of highly available nutrients and abundant microbial inoculum in the sludge. The difficulty encountered in rototilling the sewage sludge into the rocky subalpine spoil meant that much of the sludge was concentrated in the top 5 cm of the spoil. Its effect on the decay of filters would therefore have been more pronounced in the subalpine spoil than in the grassland spoil where the sludge was incorporated to at least a 15 cm depth. It is worthwhile noting, that cellulose degradation in the peat treatment was similar to that measured in the untreated spoil, although microbial biomass in the peat was significantly greater than that in the other treatments. This suggests that factors other than microbial biomass are important in determining decay rates of substrates placed on the soil surface (e.g. soil nutrient availability). Available P in the peat during the early stages of the study was excessively low (Visser et al., 1984) and this may have inhibited both plant growth and decomposition.

When the short term (12 mo) study of filters was again tested 28 mo after amendation and planting slender wheatgrass, it was observed that the cellulose decay potential of each of the treatments had not substantially altered since the initiation of the study (i.e. decay of filters in the sludge amended minespoil was significantly faster than in the peat, fertilizer and untreated minespoil). The lack of a change in decay potential implies that the growth of slender wheatgrass in the peat, fertilizer and untreated spoil had no significant effect on cellulose degradation over the term of the study. However, the more rapid decay in the sludge treated plots may have been a result of both the nutrient quality of the sewage sludge improving the chemical quality of the pure cellulose and the stimulated plant growth ameliorating the microclimate at the soil surface. Although slender wheatgrass productivity in the sludge treated spoil was much reduced after the second growing season, this did not appear to influence decay rate of filters placed in the plots at the conclusion of the third growing season. The thatch of undecomposed grass litter which had accumulated on the plots after the second growing season may have improved temperature and moisture conditions at the soil surface, in the same manner as foliage density of herbs and grasses has been postulated to do.

As in the previously mentioned studies, the highly lignified nature of the fir wood dowel resulted in negligible weight loss in all treatments over the first two years of the investigation.

4.2.4.6 Decomposition of grass and clover litter (leaves and stems). Slender wheatgrass leaves placed in the slender wheatgrass plots at the conclusion of the third growing season, lost approximately 16% of their weight over the winter and 50% after 1 yr in the field. It is highly likely that the initial weight loss was due to the disappearance of the highly soluble fraction of the litter which for Agropyron litter has been estimated at approximately 16% (see field study discussion). Although the productivity of slender wheatgrass was greatly stimulated by sewage sludge and fertilizer amendation, decomposition of the incoming litter was equally fast in all treatments. The equally fast mineralization of nutrients immobilized in the litter in plant systems of low productivity vs. plant systems of high production, would result in a greater accumulation of undecomposed litter in the highly productive systems. If more nutrients become immobilized in the litter than are available for plant growth, the consequence would be poorer plant growth or even plant failure. Also a thick litter layer can become a fire hazard, or a shelter for rodents which, if they multiply extensively, can be a menace to newly planted tree seedlings. Therefore a reclamation strategy should take into account the long-term consequences of stimulating grass/legume growth via fertilization and should perhaps include measures for reducing dead litter accumulation (e.g. grazing, harvesting) if a thick litter layer develops. It should be emphasized that the decay of slender wheatgrass was followed over a very short term. It is therefore possible that once the decomposing litter is incorporated into the soil, the mineralization and cycling of the remaining nutrients would be influenced by soil amendation. Further research is required to determine long term treatment effects on mineralization of nutrients immobilized in litter which has accumulated on minespoils.

After one year in the field, the weight remaining of slender wheatgrass stems was approximately 30% greater than the amount of slender wheatgrass leaves remaining, with no significant differences being recorded between the variously amended plots. The slower decay of the stems was probably due to a difference in resource quality between the stems and leaves. The stems may have had a wider C/N ratio, a lower soluble fraction and a higher lignin content than the leaves, thereby reducing their decay. Carrel et al. (1979), utilizing the C/N ratio of grass as an index of litter quality, concluded that C/N ratio was a major factor in determining the decay rate of litter on minespoils. Therefore in future research it may be worthwhile to follow C/N ratios and lignin contents of litter placed out on minespoils. The two different decay rates exhibited by the leaves and stems would result in two different patterns of nutrient release. This may be advantageous in soil systems which are highly leachable and where nutrient conservation is a potential problem. Conversely, the slower decay of the stems would result in slower organic matter accumulation.

Of the substrates utilized in the decomposition studies, slender wheatgrass leaves decayed most rapidly, followed by the stems and lastly the filter paper. As has been mentioned previously, natural plant litter is nutritionally much better balanced than a pure substrate such as cellulose, hence microbial growth and turnover would be expected to be more active in the litter than the filters. The comparison of the three study materials demonstrated that the decay rate of filter paper cannot be extrapolated to predict decay rates of natural litter. However, cellulose filter decomposition may be a good indicator of the levels of microbially available nutrients in minespoils which have received various amendments, since the degradation of pure cellulose is probably highly dependent on the soil nutrient conditions surrounding it.

A comparison of slender wheatgrass litter (leaves plus stems) and alsike clover litter (leaves plus stems) decay rates showed that alsike clover litter was mineralized more rapidly than grass litter. Again the difference in decay rates between the two litter types can be explained by differences in resource quality. Legume litter tends to be less "tough" than grass litter, and because legumes fix atmospheric nitrogen, the N content of clover shoots tends to be higher than that observed for grass shoots (see Table 40, Visser et al., 1984). Legume litter (particularly the leaves) may also have a larger soluble fraction than grass litter. All these factors would serve to hasten the decay of the clover litter. Since legume shoots contain more N than grass shoots, legume litter would contribute more N to the soil system when it decomposes. This would be particularly important in minespoils where N can be the nutrient most limiting to plant growth.

Lanning and Williams (1980) compared the decomposition rates of grass and clover in china clay sand wastes and, as in this study, found that clover decomposed more rapidly than grass. They also observed that nitrogen was released only from the decaying clover, but that this nitrogen did not accumulate in the sand waste. The loss of N from the sand wastes was attributed to rapid leaching of the mineralized nitrogen, loss of shoot material through wind and rain erosion and volatilization of N as ammonia at a pH above 6.5 (Lanning and Williams, 1980). The research of Lanning and Williams emphasizes the importance of gaining insight into the ultimate fate of N released from decaying plant material on minespoils. Severe leaching losses, which are possible in the coarse textured spoil such as the subalpine spoil could eventually lead to the regression of the established plant cover. The decomposition results in this study have conclusively shown that the decay and presumably the release of N from alsike clover litter is significantly faster than that of grass litter. However, the beneficial aspects of the more rapid nutrient release from this legume (i.e. faster return of N to the primary producers) would be lost if a reclamation strategy did not take into account the potential mechanisms for nutrient loss and measures for reducing this loss if it becomes excessive.

4.2.4.7 N_2 fixation potential of alsike clover. Since legume litter exhibits a relatively high turnover rate resulting in a more rapid release of essential plant nutrients and presumably a faster accretion of stable organic matter, and since the N_2 fixing capability of legumes can stimulate the rate of N accumulation, the successful establishment of legumes within a grass/legume sward appears to be an essential requirement in the initial stages of soil genesis on a minespoil. Therefore, it is necessary that we understand the effects of various amendments on the establishment and N_2 fixing capacity of legumes planted in minespoils. In this study, no significant amendment effects on N_2 fixation capacity of alsike clover could be detected, but, as previously mentioned for the rambler alfalfa, measurements were extremely variable.

The lack of acetylene reduction by alsike clover grown in the peat treatment for 3 mo was originally believed to be due to a deficiency of available P in the peat which according to Munns (1977) can limit N_2 fixation by limiting the growth of the host plant. However, a greenhouse pot experiment demonstrated that growth and flower production by alsike clover were indeed limited by low P levels, but nodule formation and N_2 fixation potential were not stimulated by additional P. Therefore, the lack of N_2 fixation was attributed to the high levels of NO_3-N (450 $\mu g\ g^{-1}$ dwt) in the peat immediately after its application. The inhibitory effects of high nitrate levels on infection, nodulation and N_2 fixation by Rhizobium have been well documented by Munns (1977). Interestingly NO_3-N levels in the peat treated minespoil dropped considerably (97 $\mu g\ g^{-1}$ dwt) after the first growing season, which may explain why acetylene reduction by alsike clover from the peat treatment increased substantially over the second growing season. It is also possible that high ammonia and nitrate levels in the sewage sludge reduced the N_2 fixing capacity of alsike clover, but that this inhibitory effect was obscured by high variability. As for the rambler alfalfa, N_2 fixation by alsike clover was highest after the second growing season. No significant relationships were detected between clover shoot N, total soil N, soil NO_3-N and the N_2 (C_2H_2) fixation capacity measured after the second growing season (i.e. 14 mo after planting). By growing alsike clover in spoil ranging from 1.67% N (peat treated) to .09% N (untreated), it is not surprising that no relationships existed between shoot N and N_2 fixation potential. The interaction between high levels of soil NO_3-N and the N_2 fixation process would make it extremely difficult to separate plant uptake of soil N from accumulation of N in the shoot as a result of N_2 fixation. The measurement of N_2 fixation by alsike clover grown in the subalpine minespoil mixed with different levels of a particular amendment would perhaps clarify some of the relationships which may exist between soil N, shoot N and N_2

fixation and more precisely determine the effects of amendation on the establishment of an N_2 fixing plant system. Although the data in this study are extremely variable, they do suggest that the physical and chemical characteristics (e.g. nutrient status) of a minespoil both before and after amendation requires some understanding to ensure the rapid establishment and maintenance of N_2 fixing legumes.

4.2.5 Microbial Development in Amended Oil Sands Tailings

4.2.5.1 ATP, microbial numbers and fungal mycelial lengths. Estimates of ATP, bacteria, actinomycetes and fungal mycelium in the sand tailings shortly after amendation and 15 mo after amendation and planting slender wheatgrass followed a similar pattern to that observed for the subalpine spoil treated with the same amendments (i.e. ATP and bacterial counts were highest in the sludge treated sand while actinomycetes and fungal hyphae were most numerous in the peat treated spoil). The similar results imply that the same factors as those discussed for the subalpine spoil were involved in determining the microbial status of the tailing sand after amendation. The main difference between the extracted oil sands and the subalpine spoil appeared to be the greater effect of amendation in the subsurface soil in the oil sands minespoil. Since the sandy spoil provided no resistance to the incorporation of the sewage sludge and peat, the microbial inoculum and nutrients present in these two amendments were dispersed to a greater depth. In general, microbial counts and hyphal measurements were substantially lower in the variously amended tailing sand than in the subalpine spoil. This would be expected since the harsh procedure used to extract oil leaves the resulting sand deficient in both nutrients and microbial inoculum. Previous microbiological investigations conducted on the oil sands tailings (Rowell, 1977; Rowell, 1978) also recorded relatively low numbers of bacteria and actinomycetes in the tailing sand, but the addition of peat and overburden led to an increase in microbial numbers.

As in the case of the subalpine spoil, the short term growth (i.e. 15 mo) of slender wheatgrass had minimal effects on soil bacteria, actinomycete counts and fungal hyphae density. The lack of a plant effect through root exudation and plant litter input can probably be accounted for by the low productivity of the grass in all but the sludge treated sand. The increase in actinomycetes in the sludge amended sand, particularly at the lower depth, may have been due to the release of large quantities of root exudate resulting from the tremendous growth of the slender wheatgrass during the second growing season. It is worthwhile noting that rototilling the sludge and peat into the sand caused such large variation in microbial population estimates, that the effects of amendation and plant growth on the soil microorganisms may have been masked by this variation.

4.2.5.2 Microbial respiration and biomass in unplanted minespoil. Although microbial respiration and biomass C were generally greater in the unvegetated subalpine spoil than in the unvegetated oil sands spoil, the effects of amendation on these two parameters over the term of the study were identical in the two spoils. That is, microbial activity and biomass C were highest in the peat treated sand followed by the sewage sludge treatment and the fertilized and untreated sand (these data are supported by the bacterial, actinomycete counts and hyphal lengths). Both respiration and microbial biomass rose significantly between 0.5 and 15 mo after treatment, but whereas respiratory activity returned to levels similar to those shortly after amendation, microbial biomass C did not. Possible explanations for these results are believed to be very similar to those discussed for the subalpine spoil. The substantial increase in CO_2 efflux and microbial biomass C measured in both the subalpine and oil sands spoil, 15 mo after treatment with peat suggests that increased biological oxidation of peat C may have been taking place, but that the oxidation occurred over a very short time span (2 years). However, measurement of organic matter by the loss on ignition method, implied that much of the C introduced into the minespoil with the sewage sludge and peat was reasonably resistant to microbial attack since no significant decreases in organic matter were detected in the 39 mo term of the study.

4.2.5.3 Microbial respiration and biomass in plots planted with slender wheatgrass or jack pine. The pattern of microbial respiration and biomass C development in the variously treated sand tailings was not significantly altered by the growth of jack pine over the four year study period (i.e. the amendments were much more important in determining microbial activity than tree growth). This is not surprising since the slow growth of a conifer such as jack pine in comparison with that of slender wheatgrass would suggest that any microbial stimulation resulting from root exudation by the pine would only occur over the long term. Also, microbial development resulting from the decay of dead plant parts would be considerably lower for pine than for the highly productive grass. Although no significant differences were detected in microbial biomass C in the sludge treatement amongst the four sampling times, there did appear to be an increasing trend in microbial biomass as time progressed. Root and shoot production by jack pine was considerably greater in the sewage sludge treatment than in the other three treatments (especially between 27 and 39 mo after planting) and therefore, may have contributed somewhat to the stimulation of microbial growth particularly during the last two years of the study. A longer term investigation would be required to determine conclusively that a relationship exists between jack pine productivity and development of soil microbial activity. Also it would be interesting to attempt to separate microbial development resulting from needle litter accumulation on the soil surface, from that stimulated by root activity.

Microbial biomass C measured 39 mo (four growing seasons) after planting tended to be higher in the 0-5 cm depth amended sand planted with slender wheatgrass than in the sand which remained unplanted or was planted with jack pine. At this time, loss on ignition was also greater in the slender wheatgrass planted plots than in the other two treatments which implies that the input of dead grass litter and roots after the second growing season was highly effective in raising organic matter levels thereby stimulating the growth of the microbial biomass. Needless to say, the unamended sand exhibited little change in loss on ignition, microbial activity and biomass over the term of the study mainly because the growth of all test plant species was negligible in this treatment (see Fig. 4, Visser et al., 1984). With the exception of the unamended controls, the patterns of plant growth, litter input and microbial development were very similar in the oil sands and subalpine minespoils. Some microbial development occurred in the unamended subalpine spoil planted with slender wheatgrass, but the paucity of nutrients in the unamended sand tailings resulted in plant failure and no microbial development. The similarity in microbial development in the two minespoils after amendation and planting is thought to be due to the linkage between plant and microbial productivity. Factors involved in this relationship have been discussed previously (subalpine spoil) and are believed to be equally relevant to the oil sands minespoil.

Between 0.5 and 15 mo after amendation, microbial activity and biomass in the fertilized sand tailings planted with slender wheatgrass rose considerably. Since the extracted sand contained very little organic carbon, the increased microbial activity in this particular treatment can be attributed mainly to C input from root exudates and sloughed root material. This again emphasizes the importance of plant growth to the microbial system, particularly in tailing sand which has not received an organic amendment. Microbial respiration and biomass in the sludge treated 0-5 cm sand peaked at 27 mo after amendation and planting grass. Nutrient release from dead plant parts and decomposing sludge would be mainly responsible for stimulating microbial activity at this time. Although slender wheatgrass in the fertilized and sludge treated sand failed to grow after the second growing season, microbial respiratory activity and biomass were not significantly reduced. The substantial input of dead litter and roots by slender wheatgrass in these two treatments presumably provided enough energy and nutrients to maintain the increased microbial biomass over the term of the study. The decreasing trend in CO_2 evolution from the fertilized and sludge treated sand towards the end of the study was also observed in the subalpine spoil and is presumed to have resulted from the same factors. Microbial activity and biomass in the upper 5 cm of the peat treated sand planted with slender wheatgrass was greater than that in the other three treatments and exhibited an increasing trend with time. This increasing trend is presumed to have been a result of slow biological oxidation of the peat, microbial stimulation by root exudates and plant litter/root input (which in contrast to the sewage sludge and fertilizer treatments did not cease after the second growing season but rather appeared to stabilize) or a combination of both these factors. The biological oxidation of newly excavated peat and the effects of plant growth on this oxidation warrants further research.

In contrast to the subalpine spoil, microbial development in the subsurface tailing sand did not demonstrate an increasing trend over the term of the study. Root production in the oil sands minespoil was considerably less than that in the subalpine spoil (the subalpine spoil prior to amendation was not as deficient in nutrients as the sand tailings) which may partially explain the lack of microbial development in the deeper sand. As mentioned previously, variation in the microbial data was considerable and this variation may have masked any changes in microbial activity.

Because microbial development appeared to be heavily dependent on the level of plant productivity, it was believed that the calculation of correlation coefficients between the products of grass growth (litter and roots) and microbial activity and biomass would demonstrate a strong relationship. However, as in the subalpine spoil, a significant relationship was not detected. Possible reasons for this are believed to be similar to those discussed for the subalpine spoil.

4.2.5.4 Decomposition of standard substrates (filter paper and wood dowel). As was observed for the subalpine spoil, amendation of the tailing sand with sewage sludge hastened the short term (12 mo) decay of filter paper but only if the spoil had been planted with slender wheatgrass. The high levels of nutrients and microbial inoculum introduced with the sewage sludge plus the possible microclimatic amelioration resulting from the stimulated grass growth during the second growing season are believed to have been the main factors in accelerating the decay of filters placed on the sludge treated sand. After spending two years in the field, no significant differences were detected between % weight remaining of filters placed in the fertilized and sludge treated slender wheatgrass plots. Since primary production of the grass in these two treatments peaked during the second growing season, the resultant litter layer which accumulated above the filters during the third growing season may have stimulated the degradation of the filters by improving both microclimate around the filters and the nutrient levels within the filters (nutrients leached out of the decaying grass litter or released from the decaying sludge may have been absorbed by the filter paper).

The lack of a significant difference in % weight remaining for filters placed in the peat and untreated plots was also observed for the subalpine spoil. Since the peat harbored a wide variety of organisms and was highly active microbiologically, the slow decay of cellulose in this treatment was attributed not to a lack of microbial inoculum, but to the low levels of P which could have retarded both plant growth and the decay process.

The importance of a vegetative cover in accelerating the decomposition of a pure substrate such as cellulose is substantiated when the decay rates of filters placed in the jack pine plots are considered. Filters placed in the fertilized and sludge treated slender wheatgrass plots decomposed much more rapidly than those placed in the jack pine plots, particularly during the third growing season. Also no amendment effects on decay rates were detected for filters placed in the jack pine plots but in the case of the sewage sludge treatment (where after 24 mo decomposition 40% of the weight remained compared with 62-78% in the other treatments), this may have been due to highly variable results.

When the 12 mo weight loss of filters was again measured 28 mo after planting slender wheatgrass, it was observed that the decomposition potential of the sludge treated sand had decreased significantly while the potential of the fertilized, peat and untreated sand had not altered significantly from that measured during the first 12 mo of the study. The reduced decomposition in the sludge treated spoil is difficult to explain since the decay potential of the sludge amended subalpine spoil did not change significantly over the 40 mo term of the study. It is possible that the development of a moss layer in the sludge treated sand planted with slender wheatgrass may have inhibited the decomposition of the filters by separating them from the nutrients being slowly released from the sludge. No such moss layer developed beneath the slender wheatgrass in the sludge treated subalpine spoil. The effects of moss layer development (particularly in recently revegetated minespoil) on the decay of incoming litter and organic matter accretion may be worthy of further research.

The highly lignified and decay resistant qualities of the fir dowel again resulted in negligible weight loss of this resource when placed in the variously amended slender wheatgrass and jack pine planted tailing sand.

4.2.5.5 Decomposition of grass and sainfoin litter (leaves and stems). Although the subalpine and oil sands minespoils were very different in terms of their physical, chemical and biological characteristics, the short term decomposition of slender wheatgrass litter was surprisingly similar in the two minespoils, regardless of treatment. Weight loss of the litter placed in the variously amended sand planted with slender wheatgrass amounted to approximately 20% over the winter (16% in the subalpine spoil) and 55% after 1 year (50% in the subalpine spoil). These data are interesting in that they imply that over the short term (i.e. 1 year of decomposition) the chemical and physical properties of a minespoil (even after amendation) and the soil microbial biomass in the soil immediately beneath the decaying litter are insignificant in determining the initial rate of decomposition of incoming dead plant material (in fact no significant relationship was detected between % dry leaf weight remaining and soil microbial activity and biomass immediately below the slender wheatgrass litter bags). It is possible that nutrients leached from the decaying litter into the soil below it may be mineralized or recycled differently depending on minespoil treatment. As mentioned for the subalpine spoil the equally fast decay rate of grass leaves regardless of treatment may have far reaching consequences in that plant growth stimulated by nutrient additions (eg. fertilizer) may result in the accumulation of a thick mat of litter which can behave as a nutrient sink, thereby reducing plant growth and perhaps plant establishment. The decomposition rates (both short and long term) of the products of stimulated plant growth should, therefore, be taken into account when determining reclamation strategy.

The weight loss of slender wheatgrass stems after 12 mo in the variously amended tailing sand planted with slender wheatgrass was also very similar to that of stems placed in the subalpine spoil (37% weight lost in the oil sands spoil vs. 33% in the subalpine spoil). The significantly slower decay of the stems compared with the leaves can be explained by a higher C/N ratio, a smaller soluble component and a higher lignin content in the stems. The potential importance of leaves and stems decaying and releasing nutrients at two different rates on nutrient conservation and organic matter accumulation on minespoils has been discussed previously. It should be emphasized that all the slender wheatgrass decomposition studies were performed after primary production by this grass had peaked. Although the bagged litter was placed below the mat of litter which had developed on the various plots, it is possible that in a system exhibiting a highly productive vegetative cover, the rates of decay would be altered as a result of microclimatic effects.

When the decomposition rates of the filter paper and slender wheatgrass leaves and stems were compared, it was observed that, with the exception of filters placed on the fertilized sand tailings, decay followed a similar pattern to that observed for the subalpine spoil (i.e. leaves degraded most rapidly followed by stems and lastly filter paper). The faster decomposition of the natural litter has been attributed to its better balanced nutrient content, which encourages the activity of the decomposer microorganisms. The problems of using filter paper decay rates to extrapolate to natural litter decomposition has already been discussed. The data collected in this study imply that the use of a pure substrate such as cellulose to predict the turnover of roots in variously treated tailing sand (Rowell, 1978) may result in an underestimate of root decay and nutrient mineralization from this compartment.

In contrast to the alsike clover leaves which decomposed more rapidly than slender wheatgrass leaves on the subalpine minespoil, sainfoin leaves and stems decayed more slowly than slender wheatgrass leaves on the variously amended tailings sand. Since the N content of the sainfoin leaves was, like that of alsike clover, greater than that in the slender wheatgrass leaves (2.9% in the sainfoin versus 2.3% in the grass litter) it is suggested that factors other than the C/N ratio controlled the decay of this particular legume. Perhaps sainfoin synthesizes polyphenolic compounds which inhibit decomposer organisms, thereby reducing its decay rate. The short term weight loss of the sainfoin and slender wheatgrass litter demonstrated that if a mixture of these two plant species was used to revegetate an area disturbed by mining, nutrient release from the aboveground litter could be expected to occur at three different rates (slender wheatgrass leaves would mineralize most rapidly, followed by sainfoin leaves plus stems and lastly grass stems). This type of pattern of nutrient release may be an advantage for minespoils in the early stages of soil genesis, where rapidly decaying litter would provide a continuous supply of nutrients required to maintain primary production, while the slowly decaying litter would contribute to the accumulation of stable organic matter.

The lack of an amendment effect on the decomposition of sainfoin litter is interesting, in that, contrary to the slender wheatgrass, sainfoin continued to grow after the second growing season. However, the greater density of sainfoin foliage in the sewage sludge and peat amended tailing sand did not significantly influence the rate of decay of dead sainfoin shoots, suggesting that neither the chemical and biological characteristics of the tailings sand nor foliage density were important in regulating the short term decomposition of litter. Further investigations are required to determine how amendation and vegetative cover affects long term decomposition and whether rates of decay of litter and cycling of nutrients back to the primary producers are significantly influenced by the development of a moss layer or burial of the litter by wind and water erosion.

4.2.5.6 N_2 fixation potential of sainfoin. The importance of the N_2 fixing legume in improving the N status of a minespoil (unless the minespoil is highly leachable) has been emphasized for both the grasslands and subalpine spoils. In these spoils, N_2 fixation by the legumes (i.e. rambler alfalfa and alsike clover) was not significantly affected by amendation but acetylene reduction measurements were so variable that any potential effects were presumably masked. Possible mechanisms to decrease the variability (i.e. processing more individual plants or even field measurements of acetylene reduction) were discussed. As in the other two studies, the N_2 fixation potential of the sainfoin at the conclusion of each of the first three growing seasons was highly variable with the highest N_2 fixation activity occurring after the second growing season. Acetylene reduction estimated after the first growing season was extremely low in all treatments. As for the alsike clover in the subalpine spoil, N_2 fixation by sainfoin in the fertilized, sludge and peat treated tailing sand may have been inhibited by high NO_3 (and possibly other forms of N) levels introduced with all three amendments. Because the tailing sand has less of a nutrient adsorptive capacity than the subalpine spoil, it is possible that much of the N released from the sludge and fertilizer immediately after their application to the sand was concentrated in the soil solution, thereby enhancing the inhibitory effects of the various forms of N on N_2 fixation by sainfoin during the first growing season.

Visser et al. (1984) presented data which suggested that much of the N added with the fertilizer and not taken up by the plants had leached out of the top 15 cm of the tailing sand by the end of the first growing season. The reduced soil N levels in the fertilized plots may account for the increased N_2 fixation exhibited by sainfoin during the second growing season. However, the inhibitory effects of sewage sludge on acetylene reduction continued through the second growing season and were significant during the third growing season. Reasons for the inhibition of N_2 fixation by sewage sludge may have been due to:

(i) long term release of high levels of NO_3 from the sewage sludge aggregates as they decomposed over the term of the study. As mentioned previously, Munns (1977) in his review of mineral nutrition and the legume symbiosis, noted that various studies have demonstrated that high NO_3 levels inhibit initial nodule development, retard nodule growth and reduce rates of N_2 fixation.

(ii) high levels of heavy metals (eg. Cd, Ni, Cu) around the sewage sludge aggregates (areas where the roots tend to concentrate due to the high nutrient status of the sludge). Vesper and Weidensaul (1978) found that 1-5 ppm of Cd, Ni and Cu reduced nodule number, N_2 fixation or both. Zn also reduced nodulation but inhibited N_2 fixation only at the 5-10 ppm levels. Copper levels in the sewage sludge treated plots were significantly greater than in the other treatments (Table 9, Visser et al., 1984), a factor which combined with the high nitrogen levels may have severely reduced N_2 fixation.

Correlation coefficients calculated between shoot N, total soil N, soil NO_3-N and N_2 (C_2H_2) fixation capacity after growing sainfoin for 14 mo revealed no significant relationships.

Identical results were obtained for the alsike clover planted in the subalpine spoil, and it is therefore believed that in the case of the sainfoin, the lack of a relationship between N fixation, and shoot and soil N status resulted from the same factors (i.e. the interactive effects of soil NO_3-N levels, N fixation and shoot N levels). To separate these effects would require detailed research to determine the role of different N levels introduced via fertilizer or sewage sludge on nodule development and N_2 fixation potential.

5. SUMMARY

5.1. FIELD STUDY

The following concluding statements summarize the data collected in the field investigations.

1. With the exception of the grasslands, disturbance by mining caused a significant decrease in the number of bacteria and actinomycetes and lengths of fungal hyphae particularly when the surface soil from the disturbed and undisturbed sites were compared. At the grasslands site, bacterial numbers were greater in the mined site while actinomycete numbers tended not to be different in mined and unmined soils.

2. Fungal hyphae appeared to be more sensitive to mining disturbance than the bacteria.

3. After mining the bacterial community shifted from one dominated by *Bacillus* and unpigmented Gram negative rods in the grassland or *Cytophaga*, *Flavobacterium* and unpigmented Gram negative rods in the undisturbed subalpine site to one dominated by coryneform bacteria.

4. Mining also caused a major shift in the composition of the fungal communities. The most frequently isolated fungi in the grasslands and oil sands minespoil were *Cladosporium* spp., *Alternaria* spp. and yeasts - organisms common in the atmosphere. In the subalpine minespoil, *Chrysosporium pannorum* and *Candida* sp. were the dominant fungi.

5. Measurements of microbial activity (CO_2 evolution), microbial biomass C and ATP were significantly less in the minespoil than in the undisturbed soil, particularly when undisturbed and disturbed surface soils were compared.

6. The general decrease in bacterial and actinomycete numbers, fungal hyphal lengths, microbial respiration, microbial biomass C and ATP as a result of mining was attributed to reduced soil organic matter levels in the minespoils.

7. The physiological technique for estimating microbial biomass C (Anderson and Domsch, 1978) appears to be the best and most

rapid method for determining the effects of disturbance on the soil microbial biomass.

8. N_2 fixation by free-living soil bacteria was negligible or non-existent in the minespoils and very low in the undisturbed sites. Evidence from the literature suggests that N accumulation in minespoils by free-living bacteria is unimportant in comparison with N inputs from N_2 fixing legumes.

9. Decay rates of fir wood dowel were so slow that differences in the decomposition of a lignified substrate between disturbed and undisturbed sites could not be detected.

10. Decomposition rates of filter paper were similar in undisturbed soils and minespoils over the short term (i.e. 12 mo) but were more rapid in the minespoils than in the undisturbed soil over the long term (i.e. 24-33 mo). Faster incorporation of the filters into the soil matrix in the minespoil thereby improving temperature/moisture conditions is a possible explanation for these results.

11. Although microbial activity and biomass were found to be lower in the minespoils than undisturbed soil, this biomass was nevertheless highly capable of rapidly decaying a pure substrate such as cellulose.

12. The adjustment of the C/N ratios of the wood dowel and filter paper with ammonium nitrate did not accelerate the decay of either of these two substrates. The quality of the C (that in wood being more resistant to microbial attack than that in cellulose) may regulate the decay rate of a substrate to a greater degree than the level of N. It is also possible that much of the added N was lost through leaching before microbial utilization, or may have been present in such a concentrated form that it was toxic to microbial growth.

13. The relationship between filter and natural litter decomposition was found to be site specific and was postulated to be dependent on litter type characteristics (i.e. resource quality), climate and the speed at which decomposing substrates are incorporated into the soil matrix.

14. Leaf litter (wheatgrass leaves or fir needles) decayed more rapidly than the woody substrates in the undisturbed grassland and subalpine sites. The high C/N ratio and high lignin content of the wood explains its decay resistance.

15. In contrast to the grasslands and subalpine sites, jack pine needle litter decomposed at the same rate as jack pine branchwood over the short term (i.e. 24 mo) at the oil sands study area. It is postulated that moisture may be the predominant variable controlling decay at this site. Until moisture conditions are more conducive to microbial growth, resource quality may be a minor factor in controlling the decay of a particular substrate.

16. Microbial activity, microbial biomass C and organic matter increased as the age of the revegetated minespoil at the subalpine site increased. Microbial biomass C was highly correlated with soil organic matter content, suggesting that it is the accumulation of organic C resulting from the decay of the products of plant growth which is the main factor influencing the rate of re-establishment of microbial activity in minespoils.

5.2 TANK STUDY

5.2.1 Prairie Grassland Minespoil

1. Of the three amendments (i.e. gypsum, sewage sludge and topsoil), sewage sludge exhibited the greatest microbial activity with the highest bacterial numbers and largest amounts of fungal hyphae. The application of the sludge to the grassland minespoil significantly increased the number of bacteria in the upper 5 cm of the spoil compared with the other treatments. By stimulating plant growth, sewage sludge may also have increased bacterial growth in the rhizosphere of fall rye during the second growing season.

2. The highly acidic nature of gypsum may have had an adverse effect on actinomycete growth in the spoil since many actinomycetes are highly sensitive to acidity.

3. Both sewage sludge and topsoil amendation were effective in improving microbial activity and biomass in the

grassland spoil. Treatment effects were located mainly in the upper 5 cm of the spoil where the bulk of each of the amendments was concentrated.

4. The CO_2 efflux in all treatments peaked when shoot production by fall rye peaked which suggests that soil microbial activity and plant productivity are closely linked. Carbon released into the spoil in root exudates and sloughed root material was postulated to be a major source of energy for stimulating microbial growth during the early stages of reclamation, particularly in spoils lacking easily available carbon. When growth by fall rye failed during the third growing season, microbial activity returned to its pre-planting levels.

5. Microbial biomass increased with increased plant productivity, and was capable of maintaining itself at a reduced metabolic level for at least one year after plant growth failed.

6. Over the first two growing seasons, decay of cellulosic filter paper placed on the spoil surface in plots planted with fall rye or rambler alfalfa was most rapid in the topsoil treatment, followed by the sewage sludge treatment and the gypsum and untreated spoil. When the growth of fall rye ceased, the decay potential of the topsoil and sludge treatments decreased to a point where no significant effects of amendation on decomposition were detected. However, in plots planted with alfalfa (which continued to grow over the term of the study) the decay potential of the topsoil and sludge treatments increased substantially as plant cover increased. It is suggested that cellulose decay potential of the grassland minespoil in the early stages of reclamation may be related to shoot density and productivity rather than the chemical and biological characteristics of the amended spoil. Increased foliage density may ameliorate microclimatic (temperature and moisture) conditions at the soil surface, thereby accelerating cellulose decay. As moisture and temperature conditions at the soil surface improve with increasing plant cover and dead litter accumulation, the nutrient quality of the litter and amended minespoil may become increasingly important in regulating cellulose decay.

7. Rambler alfalfa, capable of fixing N_2, was established in all treatments. No amendment effects on N_2 fixation were recorded, mainly because the data were extremely variable. N_2 (C_2H_2) fixation potential appeared to be greatest after the second growing season.

5.2.2. Subalpine Spoil

1. The immediate effects of amendation on the microbial status of the subalpine spoil was restricted mainly to the top 5 cm of the spoil since the rocky nature of the spoil made it difficult to incorporate the amendments (sewage sludge and peat) into the subsurface spoil.

2. Although sewage sludge introduced high levels of bacteria to the spoil, peat was the most effective amendment for increasing not only bacterial numbers but also actinomycete numbers and fungal hyphae.

3. The growth of slender wheatgrass over two growing seasons appeared to have a stimulating effect on bacterial and actinomycete numbers in the subsurface (5-15 cm) spoil but not in the surface 0-5 cm spoil. Root exudates and sloughed roots may have stimulated microbial growth in the deeper soil where C may have been more limiting than in the surface spoil. However, fungal hyphae were not significantly affected by plant growth which suggests that bacteria and actinomycetes may respond to root exudates to a greater degree than the saprophytic soil fungi during the initial stages of revegetation.

4. The higher microbial numbers measured in the top 5 cm of the peat and sewage sludge treated minespoil supported the microbial activity and biomass determinations which were also observed to be greater in the peat and sewage sludge treatments than in the fertilizer and control treatments. There was some evidence that release of nutrients from the sewage sludge and peat over the first 15 mo of the study stimulated microbial growth in the unplanted spoil but that these nutrients had been utilized by 27 mo after treatment. Loss on ignition measurements suggested that microbial

oxidation of stable organic matter from the sludge and peat treated spoil was negligible over the three year term of the study.

5. Over the first three growing seasons after amendation and planting microbial activity and biomass C development in the subalpine spoil were more stimulated by the growth of slender wheatgrass than by white spruce. The growth of slender wheatgrass was particularly effective in enhancing microbial activity and biomass in the upper 5 cm of the sewage sludge, fertilizer and unamended plots. Since slender wheatgrass has a more fibrous root system than white spruce and is more productive over the short term than white spruce, it has the ability to more rapidly improve the microbially available C status of an impoverished soil by introducing large quantities of dead plant parts and root exudates. This would be particularly important in the untreated and fertilized subalpine spoil where the supply of highly available carbon for stimulating microbial growth would be dependent almost entirely on plant growth. This suggests that the development of microbial productivity is strongly linked with levels of primary productivity mainly because the metabolic activity of microorganisms is regulated by the availability of an energy source. Unless alternative sources of carbon are applied to a minespoil, the energy required for microbial growth and maintenance would be solely dependent on plant productivity.

6. Since this study suggests that the rate of microbial development in an amended minespoil is closely associated with the type of vegetative cover, the choice of plant species and the longevity of a plant species are important factors to consider when determining a reclamation strategy. Short-lived species such as slender wheatgrass may stimulate microbial activity over the short term, but once primary production ceases microbial activity may rapidly return to its pre-planting level. The microbial biomass data collected for both the grassland and subalpine spoils suggest that biomass can maintain itself for some time after plant growth ceases (presumably as long as there is carbon available from dead plant parts). By planting a longer lived plant species such as woody

shrubs, deciduous trees or conifers, soon after a short-lived plant species dies, the regression of the microbial system to its pre-planting phase may be averted.

7. It appears that the levels of plant available nutrients introduced with an amendment determines the degree of primary production (particularly of fast growing species) which in turn regulates the rate of microbial development in a minespoil.

8. There was some evidence that fertilizer, shortly after its application, inhibited microbial activity in the upper 5 cm of the spoil. A decrease in pH or the inhibitory effects of ammonia resulting from the fertilizer application may have caused this decrease in activity.

9. Cellulosic filter paper decayed most rapidly in the sludge treated plots but, unlike the grassland spoil, filter decomposition was not significantly influenced by vegetation type (i.e. grass or white spruce). It was postulated that during the early stages of revegetation, microclimatic effects on decay rates resulting from the growth of slender wheatgrass were less pronounced than those observed for fall rye in the grassland spoil plots, because shoot production by slender wheatgrass was lower than that exhibited by fall rye.

10. Over the four year term of the study, the cellulose decay potential of the variously treated subalpine spoil planted with slender wheatgrass was not significantly altered and remained highest in the sewage sludge treated plots. The nutrients and microbial inoculum present in the sludge accumulated on the spoil surface may have accelerated the decay of the cellulose by improving the nutrient quality of the paper and providing microorganisms highly active in cellulose decay.

11. Although the productivity of slender wheatgrass was greatly stimulated by sewage sludge and fertilizer amendation, decomposition of the incoming litter (both stems and leaves) was equally fast in all treatments. This resulted in a greater accumulation of undecomposed litter (and greater nutrient

immobilization) in the highly productive treatments than in the treatments exhibiting low productivity.

12. Slender wheatgrass stems decomposed at a slower rate than the leaves, resulting in two different rates of nutrient release.

13. A comparison of the decay rates of slender wheatgrass stems and leaves and filter paper demonstrated that the decay rates of filters could not be extrapolated to predict decay rates of litter.

14. Alsike clover leaves and stems exhibited a faster decomposition rate than slender wheatgrass leaves and stems. The higher N content in the shoots of the N_2-fixing alsike clover than in the slender wheatgrass shoots may have been one factor which hastened the decay of the legume litter.

15. As was the case for the rambler alfalfa in the grassland spoil, no significant amendment effects on N_2 fixation capacity of alsike clover could be detected. Again N_2 (C_2H_2) measurements were excessively variable. During the first growing season alsike clover in the peat amended spoil demonstrated no N_2 fixation ability and this was attributed to high NO_3 levels in the peat which inhibited infection, nodulation and N_2 fixation by Rhizobium. No significant relationships were detected between clover shoot N, total soil N, soil NO_3-N and N_2 (C_2H_2) fixation capacity measured after the second growing season.

5.2.3. Oil Sands Spoil

1. The immediate effects of amendation on the microbial status of the tailing sand was very similar to that observed for the subalpine spoil i.e. peat amendation introduced more microbial inoculum (bacteria, actinomycetes and fungal hyphae) to the sand than the other amendments.

2. The microorganisms and nutrients contained in the peat and sewage sludge amendments were incorporated to a deeper depth in the tailing sand than in the rocky subalpine spoil.

3. As in the subalpine spoil, short term growth of grass had minimal effects on microbial numbers and hyphal lengths in the surface spoil layer. Actinomycetes did increase in the 5-15 cm deep sludge-treated sand where profuse root production by slender wheatgrass may have stimulated actinomycete growth.

4. Although microbial respiration and biomass C were generally greater in the unvegetated subalpine spoil than in the unvegetated tailing sand, they were similarly affected by amendation in the two spoils over the term of the study i.e. microbial activity and biomass were greater in the peat and sewage sludge treatments than in the fertilized and unamended sand. Both the peat treated subalpine and oil sands minespoils exhibited increases in CO_2 efflux at 15 mo after amendation suggesting that the oxidation of peat C may have been stimulated at this time. However, loss on ignition measurements demonstrated no massive loss of stable organic matter over the four year study.

5. With the exception of the unamended sand where the paucity of nutrients resulted in plant failure and no soil microbial development, the patterns of plant (slender wheatgrass and jack pine) growth, litter input and microbial development in the subalpine and oil sands spoils were very similar. Jack pine, like white spruce, had negligible effects on soil microbial activity, while the growth of slender wheatgrass generally stimulated microbial growth and productivity in the 0-5 cm deep amended sand. These results support the premise that there is a strong relationship between plant and microbial productivity, particularly in C limited soils such as the fertilized tailing sand in this study where increases in microbial activity and biomass were mainly a result of the products of plant growth (i.e. root exudates, dead plant litter).

6. As in the subalpine spoil, the nutritional quality of the amendments determined the degree of primary production by slender wheatgrass which in turn appeared to regulate microbial development, particularly in the fertilizer and sewage sludge treatments. Microbial activity and biomass in the peat treated sand planted with slender wheatgrass demonstrated an increasing trend with time, but it

was difficult to determine whether this was due to the slow biological oxidation of the peat, microbial stimulation by root exudates and plant litter/root input or an elevated loss of peat C resulting from the input of root exudates (i.e. "priming effect").

7. The link between microbial development in a spoil and plant productivity emphasizes the importance of maintaining a productive plant cover on a minespoil.

8. In the first two years after amendation and planting, the decomposition of filter paper was fastest in the sewage sludge and fertilizer treated sand planted with slender wheatgrass. The higher cellulose decay potential of these two treatments was attributed to improved nutrient conditions in the sand surrounding the filters, introduction of cellulose decay microorganisms in the sewage sludge and amelioration of microclimate resulting from a greater foliage density and a thicker litter layer than that observed in the peat and control treatments.

9. Low levels of P in the peat may have reduced rates of cellulose decay in this treatment in both the oil sands and subalpine spoils.

10. Over the long term (four years) the cellulose decay potential of the sludge amended sand decreased significantly from that observed over the first two years after planting while decay rates in the other treatments were not significantly altered. The development of a moss layer in the sludge treated plots may have adversely affected decay rates of the filters.

11. The short term decay rates of slender wheatgrass litter measured three years after amendation and planting were not significantly affected by treatment and were approximately the same in the subalpine and oil sands spoils. These results imply that, during the early stages of revegetation, the chemical and physical properties of a minespoil and the microbial biomass in the soil immediately beneath the decaying litter play a minor role in determining the initial rate of decomposition of incoming dead plant material. For this reason, excess fertilization may stimulate plant growth but may not necessarily accelerate litter decay resulting in

the accumulation of litter on the spoil surface which behaves as a nutrient sink, and a protective cover for rodents.

12. As was the case for the subalpine spoil, slender wheatgrass stems lost less weight than the leaves after 12 mo in the field. Also, filter paper decomposition rates were not very comparable to those measured for grass leaves or stems.

13. Sainfoin leaves and stems decayed more slowly than slender wheatgrass leaves and stems. Also amendation did not significantly affect the decomposition rates of sainfoin litter.

14. Like the rambler alfalfa in the grasslands spoil and alsike clover in the subalpine spoil, sainfoin exhibited no significant amendment effects on N_2 fixation over the first two growing seasons. The N_2 fixation potential of sainfoin over the first growing season was extremely low in all treatments and this was attributed to inhibition by high levels of NO_3-N introduced with the fertilizer, peat and sewage sludge. Significant inhibitory effects of sewage sludge on N_2 fixation by sainfoin were recorded after the third growing season. Long term release of NO_3-N from the decomposing sewage sludge clumps and/or high levels of heavy metals (e.g. Ca, Ni, Cd) around the sludge clumps may have caused a reduction in nodule formation and N_2 fixation. No significant relationships were detected between sainfoin shoot N, total soil N, soil NO_3-N and N_2 (C_2H_2) fixation measured after the second growing season.

6. RECOMMENDATIONS FOR FURTHER RESEARCH

1. More research is required to determine the rates at which N is accumulated on minespoils via N_2-fixing plant species since these plants provide a less expensive and more long-term alternative to maintenance fertilizer applications.

2. It is necessary to obtain information on N mineralization rates from decaying residues of N_2-fixing species and from soil amendments under field conditions since this N supply will determine the maintenance of a specific vegetative cover. The N requirements of various plant species currently being used for reclamation purposes should also be established to determine if slow-growing species would benefit from legume nurse crops.

3. Microbial oxidation of C from amendments such as peat and the influence of plant growth (particularly fast-growing grass and herbaceous species) on this oxidation requires clarification.

4. More data are required regarding long-term decay rates of plant residues (both above- and below-ground) and the rates at which plant-available nutrients are released from these residues. Although the continual addition of nutrients may stimulate plant growth, decomposition rates of incoming plant residues are not stimulated to the same degree resulting in a thatch of litter which behaves as a slow-turnover nutrient sink. Thus effects of different management procedures such as grazing, cropping, burning and plowing in plant residues, on plant litter decay and maintenance of a vegetative cover should also be studied.

5. Data from this study suggest that spoil and amendment characteristics play a minor role in the short-term decomposition of above-ground plant residues such as leaf and stem litter. It would be profitable to define more clearly the effects of different spoil types and amendment combinations on the degradation of both above- and below-ground plant residues. It is possible root decay is much more influenced by spoil/amendment characteristics than is litter deposited on the spoil surface, particularly over the short term (12 mo).

6. This study concentrated on the effects of single amendments on individual plant species. Additional research addressing the effects of combinations of amendments (e.g. peat/fertilizer; sewage sludge/peat) on plant growth (different species) and decomposition rates of plant residues would be useful.

7. Sewage sludge, applied at low rates, has the potential for greatly enhancing plant growth over a relatively long time (5 years) on a variety of minespoils as demonstrated in this study. However, there is some indication that it may be inhibitory to such essential processes as symbiotic N_2-fixation. Further study on the inhibitory effects of sewage sludge on plant growth, N_2-fixation and decay rates is necessary. This research should be coupled with studies on N mineralization from the sludge since ammonium-N may be a major cause of toxicity in sludge-treated soils.

8. On minespoils, where agricultural crops are not the final land use goal, it may be advantageous to establish a short-lived, fast-growing plant species (fertilized only once) to provide erosion control and then replace this cover with long-lived, slower-growing woody shrubs and trees once the initial cover dies. It would be interesting to determine if the nutrient sink created by the fast-growing species would decay rapidly enough to provide sufficient nutrients for the establishment of the slower-growing species.

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REINSTATEMENT OF BIOLOGICAL ACTIVITY IN SEVERELY DISTURBED SOILS: EFFECTS OF DIFFERENT AMENDMENTS TO THREE DIFFERENT MINESPOILS ON SELECTED SOIL PHYSICAL AND CHEMICAL PROPERTIES AND ON PLANT GROWTH

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ABSTRACT

Three different minespoils (prairie grassland, subalpine and extracted oil sands) were each treated with three different organic or inorganic amendments and then planted with four different plant species. Amendation effects on some of the soil chemical characteristics and on plant growth were then monitored over 2 and 3 years respectively.

Application of topsoil was most effective in reducing the high sodium adsorption ratio characteristic of the prairie minespoil. It also promoted the best growth by rambler alfalfa. Fall rye and crested wheat were most productive in the sewage and topsoil treated spoil, while russian wild rye was most stimulated by the sewage sludge treatment. Gypsum was observed to be the least effective of the amendments tested. Crested wheat, russian wild rye and rambler alfalfa, once established, behaved as long-term cover crops, but fall rye was found to be short-lived (2 years).

The poor fertility status of the subalpine and oil sands spoil was most improved over the long term by the addition of sewage sludge. The slender wheatgrass, alsike clover, white spruce and willow planted in the subalpine soil, and the slender wheatgrass, sainfoin, jack pine and bearberry planted in the extracted oil sands were generally most productive in the sewage sludge treated spoils. The slow growing woody perennials (white spruce, bearberry) also performed well in the peat amended spoils, but low extractable P levels in the peat may have suppressed growth by the faster growing species. Mineral fertilizer stimulated shoot production by the grass, clover and willow in the subalpine spoil, but ellicited a poor response from plants grown in the tailing sand. Much of the fertilizer applied to the sand had leached out of the rooting zone by the end of the first growing season. Slender wheatgrass followed the same growth pattern as fall rye and did not reseed readily. Heavy metals introduced with the sewage sludge were not significantly concentrated in plant tissue over the short term. The N_2-fixing legumes (clover and sainfoin) were observed to be least sensitive to low soil N levels.

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1. INTRODUCTION

Mining for energy resources such as coal and oil generally results in large tracts of land lacking vegetation. Rapid revegetation of such areas is necessary: 1) to provide erosion control, 2) to restore the land to a productive state, and 3) to improve the visual quality of the disturbed land. Vogel (1981), in his guide for revegetating coal minespoils in the eastern U.S., outlined what he considered to be the main soil characteristics influencing successful vegetation establishment. They included:

1. Chemical properties:
    - i) soil pH
    - ii) acid induced toxicities
    - iii) nutrient deficiencies.

2. Physical properties:
    - i) particle size distribution
    - ii) bulk density
    - iii) steepness of slope
    - iv) erosion potential
    - v) color of soil (dark-colored soils can result in high surface temperatures which may be lethal to seedlings and cause soils to dry out more rapidly)
    - vi) aspect (whether the slope faces south, west, north or east).

3. Biological properties:
    - i) decomposer microorganisms - ultimately important in recycling nutrients (for uptake by higher plants).
    - ii) symbiotic microorganisms, such as N_2 fixers which improve the N status of legumes, alder etc., and mycorrhizal associations of various types which are believed to improve plant survival and growth.
    - iii) soil fauna which are considered regulators of the rates of organic matter decomposition (and hence of nutrient cycling)

by comminution of plant debris, by consumption of various types of organic matter and by dispersal of microbial inoculum in the soil system.

According to Bradshaw et al. (1978) there are three main steps involved in the rehabilitation of land disturbed by mining.

1. The restoration of soil fertility and structure - aim to overcome adverse physical, nutritional and toxicity factors.

2. The establishment of plant species through natural colonization or controlled planting programs.

3. The subsequent management for ecosystem development - once vegetation has been established the system can be left untended to develop, but this is usually a very slow process. To accelerate ecosystem development on derelict land, some management (such as fertilizer additions, reseeding, planting different species of plants) related to the ultimate land use is desirable.

Many minespoils are relatively infertile and may have toxicity (e.g. acidic coal minespoils in eastern U.S.) or textural problems (e.g. sodic clay minespoils of the Northern Great Plains region of the U.S.). They are usually microbiologically impoverished lacking both decomposer and symbiotic (both mycorrhizal and nitrogen fixing) inoculum. These factors generally result in unsuccessful plant establishment and poor plant growth. To overcome the problems of soil fertility and poor soil structure, most minespoils are ameliorated with inorganic (e.g. fertilizers, lime, gypsum) or organic (e.g. sewage sludge, peat, topsoil, mulches) amendments. The spoils are contoured in such a way that the susceptibility to erosion of the site is minimized. The amendments are essential for plant establishment, survival and growth in that they can improve soil structure, provide nutrients, and also serve as a source of microbial inoculum. Ideally the type of amendment, or combination of amendments, chosen to ameliorate a minesite would have all the chemical, nutritional and biological properties required to establish and sustain a vegetative cover which would develop into a productive ecosystem with a minimum of additional management. It has become well-recognized that efficient, balanced functioning of soil organisms (through their action in organic matter decomposition, nutrient cycling and symbiotic relationships to higher plants) is essential for ecosystem development and maintenance. As a result research was initiated which had the following objectives:

1. to study primary production of test plant species in various types of minespoil which had been previously ameliorated with organic or inorganic amendments or left unamended.

2. to provide detailed information on the rates of redevelopment of biological activity in various minespoils with and without soil amendments (using rates of increase in microbial biomass and species diversity of fungi and bacteria together with rates of increase in total soil metabolic activity as the indices of redevelopment).

3. to provide data on the types of microorganisms associated with the roots of plants grown in the test minespoils under the range of experimental treatments and to provide survey data on the speed of mycorrhiza formation and (where applicable) nodule formation on test plant roots.

4. to present conclusions and recommendations on the methods of rapid re-instatement of productive soil-plant systems on minespoils.

This report deals primarily with the results obtained on the effects of various amendments of three minespoils on some soil chemical and physical factors, plant growth, and nutrient levels in the foliage of selected plant species after one or two growing seasons.

It should be noted that, in this study, the aim was to investigate in detail the effects of single amendments and not to consider the effects of various combinations of amendments.

2. MATERIALS AND METHODS

The three areas chosen for the study were a shortgrass prairie site which had been mined for coal in the early 1950's and subsequently abandoned (Bow City, Alberta); a subalpine boreal forest site which is currently being mined for coal (Cardinal River, Alberta) and a boreal forest site which is currently being mined for oil sands (Suncor site, Fort McMurray, Alberta). The approximate locations of the three sites and the vegetation and soil types both before and after mining disturbance are presented in Table 1.

2.1. FIELD STUDY

At each study site, five sampling plots, each 5m x 5m, were laid out in a 15 x 15 m area in areas disturbed by mining and also in adjacent undisturbed areas (with the exception of the oil sands site where due to the inaccessibility of the disturbed areas, plots were established only in an undisturbed jack pine woodland). At each site, five soil cores were removed from the disturbed and undisturbed plots. The undisturbed plot cores were separated into organic and mineral layers while the disturbed cores (which showed no horizon development) were separated into 0-5 cm and 5-15 cm depths. Samples were sieved (< 2 mm) and air dried. To determine the effects of disturbance on selected soil chemical and physical parameters, subsamples from each depth from each site were bulked and tested for sodium adsorption ratio (SAR), organic and CO_3-C, total N, P, Fe, Ca, Mg, Na. The SAR was calculated from the soluble Ca, Mg and Na concentrations (me l^{-1}) in saturated soil extracts, i.e.:

$$SAR = \frac{Na}{\sqrt{1/2\ (Ca + Mg)}}$$

as described by Bower and Wilcox (1965). Organic carbon was calculated by substraction of CO_3-C from total C determined by dry combustion using an induction furnace (Allison, Bollen and Moodie, 1965; Allison and Moodie, 1965); CO_3-C was determined by a pressure-calcimeter method (Allison and Moodie, 1965). Total N was determined by a semi micro-Kjeldahl method employing a Hg catalyst (Bremner, 1965), and the total nutrients were determined by an acid digestion ($HNO_3/HClO_4$) procedure and atomic absorption

Table 1. Summary of vegetation and soil types at each study site.

Site	Location	Vegetation		Soil type	
		Before	After	Before	After
Prairie grassland	Bow City, Alberta	mixed grass prairie (*Agropyron*, *Bouteloua gracilis*, *Artemisia cana*, *A. frigida*, *Mamillaria vivipara*)	*Kochia scoparia*	Brown chernozem	saline-sodic, sandy clay loam (56% sand 22% clay, 22% silt)
Subalpine Boreal	Luscar, Alberta	spruce/fir forest (*Picea engelmanii*, *Abies lasiocarpa* with *Hylocomium splendens*, *Empetrum nigrum*, *Ledum groenlandicum* understory)	- (seeded with timothy, bluegrasses, fescues, wheatgrasses)	Brunisol	Regolith
Boreal Forest (Oil sands)	Ft. McMurray, Alberta	- jack pine/lichen woodland (*Pinus banksiana*, *Cladina mitis*) - black spruce/tamarack peatland (*Picea mariana*, *Larix laricina*)	- (seeded with grasses, legumes)	Brunisol	Sand

spectroscopy. Many of the methods used for these analyses have been conveniently summarized by McKeague (1976). Soil pH was determined electrometrically using 10 g soil in 20 ml distilled water.

2.2. TANK STUDY

2.2.1. Set-Up

In order to monitor more closely the effects of the variously amended minespoils on plant growth and associated soil biological, chemical, and physical factors, it was decided that a major part of the research be carried out in Calgary rather than at the more inaccessible field sites. Large quantities of minespoil from the grassland, subalpine and oil sand sites were transported to Calgary and each placed into separate plywood frames (soil tanks). A plan of a soil tank and its dimensions are presented in Figure 1). Each tank was constructed with internal dividing walls to prevent mixing of amendments at the boundaries between plots. Only the first 12 plots in each tank were used in the study because not enough spoil was available to set up 16 plots. The minespoils were divided amongst the plots and levelled so that there was approximately a 60 cm deep layer of soil per plot. Amendments were then applied to allow three replicate plots per treatment (i.e. type of amendment). Three unamended control plots were also set up for each of the three spoils. The amendments tested on each of the spoil types, their origin, application rates and application procedures are presented in Table 2. Some of the chemical and physical characteristics of each amended spoil were determined using techniques described previously. Loss on ignition was measured by placing a sieved, pre-dried (105°C) soil sample in a muffle furnace, igniting it at 375°C and leaving it for 24 h. Weight loss of the sample was then determined.

After amendation but immediately prior to planting, one large (2-3 kg) soil sample was randomly removed from two depths in each of the plots to determine the immediate effects of amendation on pH, E.C., extractable cations, SAR, total N, NO_3-N, extractable P and DTPA extractable metals. The pH, extractable cations, SAR and total N were determined by the same methods as those described for

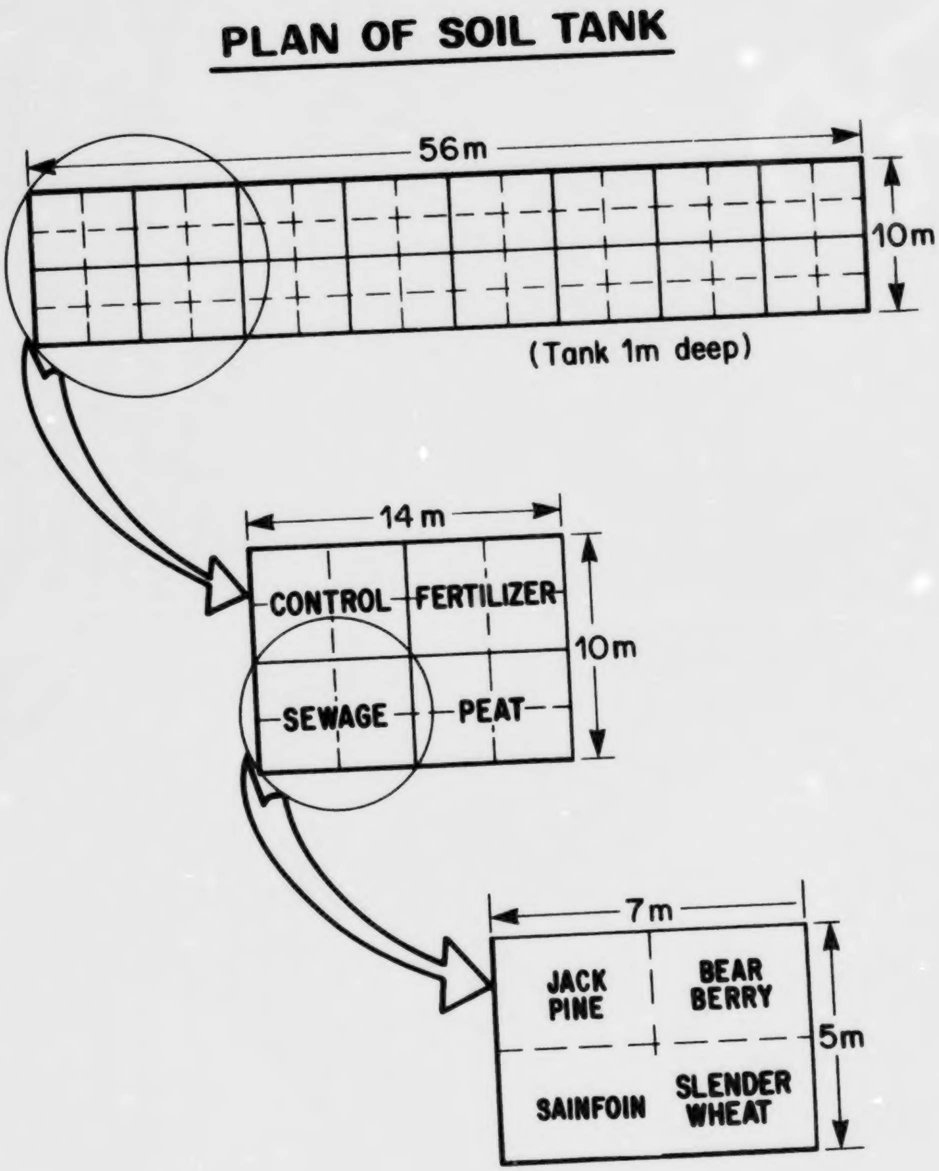
PLAN OF SOIL TANK
56m
10m
(Tank 1m deep)
14 m
10m
CONTROL
FERTILIZER
SEWAGE
PEAT
7m
5m
JACK PINE
BEAR BERRY
SAINFOIN
SLENDER WHEAT

Figure 1. Plan of soil tank.

Table 2. Details of the amendments applied to each spoil type.

Spoil Type	Amendment	Application Rate	Application Procedure
Prairie Grassland	Topsoil from Bow City grassland	15 cm layer	Spread evenly on spoil surface
	Gypsum	28.6 mT ha^{-1} (dry) (calculated to replace Na down to a 15 cm depth)	Spread on spoil surface; then rototilled to 15 cm depth
	Anaerobically digested sludge from Calgary, Alta. sewage lagoons.	46 mT ha^{-1} (dry)	Wet sludge spread on soil surface, allowed to dry, then rototilled to 15 cm depth.
Subalpine boreal forest	Feather moss peat from under spruce forest at Canmore, Alta.	15 cm layer	Evenly spread on soil surface, then rototilled into spoil.
	NPK fertilizer (23-23-0, 0-0-62)	112 kg N ha^{-1} 112 kg N P_2O_5 ha^{-1}	Spread by hand immediately prior to planting
	Sewage sludge (as above)	Same rate as for prairie grassland	Same procedure as for prairie grassland
Boreal Forest (Oil Sands)	Peat (as above)	Amendments applied at the same rate and in the same manner as that described above for the subalpine forest site.	
	Fertilizer (as above)		
	Sewage Sludge (as above)		

the field study samples. The electrical conductivity (E.C.) of the saturated soil extract was measured by the technique of Bower and Wilcox (1965). The depths sampled were 0-5 cm and 5-15 cm except for the peat and topsoil amended plots where 0-5 and 15-25 cm (spoil beneath amended soil) were sampled. Nitrate nitrogen was determined by extraction in .01 N $CuSO_4$ + .007 N Ag_2SO_4 solution and subsequent measurement by a phenol disulphonic acid colorimetric procedure (Bremner, 1965). Extractable P was measured by extraction in a .03 N H_2SO_4 + .03 N NH_4F solution and subsequent determination by an ascorbic procedure (John, 1970; Miller and Axley, 1965). DTPA extractable metals were extracted in 0.005 M DTPA, 0.01 M $CaCl_2$, 0.1 M TEA, pH 7.3 and determined by atomic absorption spectroscopy (Lindsay and Norvell, 1969). Total N, NO_3-N and PO_4-P were measured again 4 months after the application of fertilizer to the subalpine and oil sand minespoils in an effort to determine leaching losses of these nutrients after one growing season. The above-mentioned chemical and physical parameters were also assessed two growing seasons after planting fall rye in the prairie grassland minesoil and two growing seasons after planting slender wheatgrass in the subalpine and oil sand spoils.

2.2.2 Planting

After amendation each plot was subdivided into four subplots with 0.5 m walkways between them and each amended subplot was then planted with a grass, herb shrub or tree species resulting in four test plant species per plot (see Fig. 1 for the layout of one of the sewage sludge treated oil sands plots). The species planted in each of the test minespoils are listed in Table 3. Grasses and legumes were seeded by hand with each plot receiving known weights of seed. The planting rates and percentage germination of the grasses and legumes are presented in Table 4.

Spruce and pine were planted with 20 cm spacing in 14 rows of 8 seedlings each resulting in approximately 112 seedlings per subplot. Jack pine (grown by Northern Forest Research Centre, Canadian Forest Service, Edmonton) and white spruce were grown in

Table 3. Species planted in each type of minespoil.

Spoil Type	Plant Species Common Name	 Scientific Name
Grassland coal mine	Russian Wild Rye	Elymus junceus Fisch.
	Crested Wheat	Agropyron cristatum (L.) Gaertn.
	Fall Rye	Lolium perene L.
	Rambler Alfalfa	Medicago sativa L. var. Rambler
Subalpine coal mine	Slender Wheatgrass	Agropyron trachycaulum (Link) Malte
	Alsike Clover	Trifolium hybridum L.
	White Spruce	Picea glauca (Moench.) Voss
	Willow	Salix sp.
Extracted oil sand	Slender Wheatgrass	Agropyron trachycaulum (Link) Malte
	Sainfoin	Onobrychis corniculatus L.
	Jack pine	Pinus banksiana (Lamb
	Bearberry	Arctostaphylos uva-ursi (L.) Spreng

Table 4. Planting rates and % germination of grasses and legumes.

Spoil Type	Plant Species	Amount seed sown (g/m^2)	No. seeds/g	% germination (lab conditions)
Grassland	Russian Wild Rye	1.24	409	94
	Crested Wheat	1.79	439	61
	Fall Rye	10.39	47	98
	Rambler Alfalfa	1.10	508	85
Subalpine	Slender Wheatgrass	2.25	263	81
	Alsike Clover	0.44	1333	82
Oil Sands	Slender Wheatgrass	2.25	263	81
	Sainfoin	16.39	37	79

peat moss in 47 cm^3 and 192 cm^3 Spencer-Lemaire containers respectively for 20 weeks prior to planting. Willow and bearberry (purchased from the Edmonton nurseries) were planted with 25 cm spacing in 13 rows of 7 seedlings each resulting in 91 cuttings per subplot. The willow cuttings were grown in 95 cm^3 cylinders of peat moss while the bearberry cuttings were rooted in pots containing 100 cm^3 of a peat-sand mixture. All seeding and planting was effected in the last two weeks of June. Plots were kept free of weeds over the term of the study.

2.2.3. Primary Production Asessments

2.2.3.1. Grasses and legumes. Shoot production of the grasses and legumes was assessed at the conclusion of each of the first three growing seasons in the following way:

i) at the end of the first growing season (September), five intact grass or legume plants were removed from each of the three replicate subplots of each amendment in each spoil type. Shoots were separated from roots and dried in paper bags at 80°C for one week and weighed. Shoot production values on a m^2 basis were determined by multiplying the average dry shoot weight (g) per plant per amendment by the density of each plant species per subplot. The densities of each grass or legume species in each subplot had been determined four weeks after seedling emergence by counting the number of plants occurring in each of three, 25 cm x 25 cm random quadrats in each subplot.

ii) At the conclusion of the following two growing seasons (September) shoot production was assessed by means of randomly clipped quadrats. All shoots in each of three randomly placed quadrats (25 x 25 cm) in each subplot were clipped approximately 1.28 cm above the soil surface resulting in 9 replicate quadrats per treatment per sample time. Shoots were dried and weighed as described in (i) above and weights converted to a g m^{-2} basis.

Much of the legume and slender wheatgrass shoot material clipped after the third growing season was dead litter (mainly stems) from the previous year. The dead litter was separated from the live material, dried and weighed.

iii) Root production for the fall rye, slender wheatgrass and the legumes was estimated after the second growing season. Two soil cores (6 cm wide x 14 cm long) were randomly removed from each subplot and roots were washed out using 2 and 0.5 mm sieves. Roots washed out on the 2 mm sieve were picked off, dried at 80°C and weighed while the fine roots on the 0.5 mm sieve were floated out, removed from the water and dried. The dry weight of roots per soil core was then extrapolated to g dry root m^{-2} to a depth of 14 cm.

2.2.3.2 Trees and Shrubs.

i) Shoot weight was determined at the conclusion (September) of each of the first three growing seasons for jack pine, bearberry and willow and four growing seasons for white spruce. At each sampling time five plants were clipped (at the soil surface) in each plot, dried (at 80°C) and weighed. Results were expressed as g dry shoot per plant.

ii) Total root weights were measured at the end of the second growing season. Willow roots were not sampled due to the inability to distinguish individual root systems. The roots of the other woody plants were sampled by digging at points midway between plants to a depth of 15 cm in the subalpine minespoil and 25 cm in the oil sands. Roots were washed out of the soil, dried at 80°C, weighed and expressed as g dry root per plant. Plant height was measured at each sample time and survival was measured for the first two years. Dieback of willow tops was determined by measuring total height and total live height of five random plants per plot. Winter injury to bearberry was evaluated by making visual estimates of five randomly selected plants per plot. Dieback of both willow and bearberry was measured at the start of the third growing season.

2.2.3.3 Foliage chemical analysis. Elemental concentrations (P, K, Ca, Mg, Mn, Cu, Zn and Fe) were determined for the foliage of slender wheatgrass, sainfoin, jack pine (needles only), and white spruce (needles only), harvested from each plot at the conclusion of the first growing season. The methods of determination were similar to those described for nutrient analysis of the spoils after grinding the samples in a Wiley mill to pass 40 mesh screen.

Total N (Bremner, 1975) was determined for slender wheatgrass, alsike clover, willow, jack pine and sainfoin foliage collected after the second growing season since there was not enough plant sample for this analysis after the first growing season.

3. RESULTS

3.1 SOIL CHEMICAL CHARACTERISTICS

3.1.1 Field Study

The effects of mining disturbance on selected chemical characteristics of the soils under investigation are presented in Table 5. The data show that:

i) the pH of the prairie grassland soil dropped slightly after mining, whereas the pH of the subalpine and oil sands soils increased dramatically. The use of caustic soda to remove oil from the sand is presumably the main reason for the pH increase in the extracted sand tailings.

ii) the Na adsorption ratio (SAR) increased significantly at the prairie grassland site after mining. The high clay content and Na level of this minespoil explain the high SAR. The SAR and Na levels were not affected by mining at the subalpine forest site.

iii) as a result of stripping the organic mat from the subalpine site when mining, the organic C level in the resultant spoil was very low. The organic C levels measured in both the grassland and subalpine forest minespoils should be viewed with caution since these analyses were hampered by the presence of coal particles which were no doubt included in the C analyses. The extraction procedure used to remove oil from oil bearing sand results in what appears to be pure sand devoid of organic carbon and all other nutrients.

iv) the removal of the organic mat during mining at the subalpine site resulted in a decrease in total soil N; whereas the % N in the grassland site was not affected.

v) concentrations of P and Fe were greater in the minespoil than in the undisturbed soil at the subalpine site. There was virtually no P present in the oil sand tailings. Levels of Ca and Mg decreased after mining at the grassland site while mining the subalpine site resulted in higher soil Mg levels.

Therefore the main effects of mining disturbance at the three study sites were:

Table 5. Chemical characteristics of test soils before and after disturbance.

Site and Soil	pH	SAR	C(%)		N(%)	Total nutrient elements (μg g^{-1})				
			organic-C	CO_3-C		P	Fe	Ca	Mg	Na
Prairie Grassland										
Undisturbed topsoil (0-15 cm)	7.5	0.2	1.8	1.0	0.2	1412	15400	26152	10154	230
Undisturbed mineral (15-25 cm)	7.3	0.2	1.7	1.3	0.2	1003	15030	37775	11005	230
Disturbed mineral (0-5 cm)	6.9	19.5	6.4	0.1	0.2	1188	17925	4990	3539	4485
Disturbed mineral (5-15 cm)	6.8	19.0	5.1	0.0	0.2	1934	16550	4990	3034	4830
Subalpine Boreal Forest										
Undisturbed organic	4.5	0.1	31.3	0	1.0	449	7140	6583	1690	230
Undisturbed mineral	4.2	0.5	4.0	0	0.2	508	9500	661	1222	460
Disturbed mineral (0-5 cm)	7.0	0.2	3.5	0	0.1	1254	30025	3127	5168	345
Disturbed mineral (5-15 cm)	7.3	0.3	10.9	0.2	0.2	845	24925	7074	4499	345
Boreal Forest - Oil Sands										
Undisturbed organic (0-5 cm)	5.1	NM	37.6[1]	NM	NM	NM	NM	NM	NM	NM
Undisturbed mineral (5-15 cm)	6.0	NM	0.6	NM	NM	NM	NM	NM	NM	NM
Disturbed mineral (0-5cm) }tanks	8.7	NM	0.4	NM	0	1.2	2.6	NM	NM	NM
Disturbed mineral (5-15 cm) }tanks	8.7	NM	0.2	NM	0	1.0	3.2	NM	NM	NM

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[1] % loss on ignition determined for Oil Sands samples.

[2] NM = not measured.

i) higher SAR values (high Na and clay contents) and slightly decreased pH at the grassland site;

ii) decreased C and N levels and increased Fe concentrations at the subalpine site; pH also increased;

iii) increased pH and the stripping of virtually all C and other nutrients from the sandy soil at the oil sands site.

3.1.2. Tank Study

3.1.2.1 Characteristics of the amendments. The amendments applied to the various minespoils had the following chemical and physical characteristics (see Table 6a):

i) levels of organic matter, N, P were generally greater in the sewage sludge than in the other amendments. Heavy metal concentrations (particularly Zn) were also higher in the sludge than in the other amendments.

ii) gypsum (which is the mineral material resulting from the H_2SO_4 extraction of phosphate from phosphate rock) is mainly $CaSO_4$ and was therefore high in Ca. Phosphorus levels also tended to be high.

iii) prairie grassland topsoil when compared with the grassland minespoil (see Table 5) had higher biologically available organic matter levels and a much lower SAR.

iv) the peat used in this study was a fibrous feather moss peat high in organic matter pH and N. Peat samples from the 20-30 cm, 50-60 cm and 80-90 cm depths of the bog from which this amendment originated had NO_3-N levels of 271 (104-468), 424 (270-630) and 385 (44-714) $\mu g\ g^{-1}$ respectively). However amounts of P (both total and extractable) were found to be limiting to plant growth during the first growing season after amendation.

In terms of nutrient organic matter addition to impoverished minespoils, it would appear that sewage sludge would offer the best possibilities. However, the high levels of ammonium N and heavy metals should be taken into consideration when applying sludge as a soil amendment.

Table 6a. Some chemical and physical characteristics of amendments.

Amendment	pH	Loss on ignition (%)	N (%)	Total nutrients ($\mu g\ g^{-1}$)						
				P	Ca	Fe	Cu	Zn	Pb	Cd
Sewage Sludge	8.6	50.0 (30)*	3.9	15325 (170)**	44800	11950	815	1525	788	12
Gypsum	3.0	2.4 (.1)	.01	5983 (198)	19.9 (%)	255	3	41	8	6
Topsoil	8.3	7.9 (4.3)	.18	1412 (4)	26152	15400	13	69	20	<0.5
Peat	7.2	70.0 (36)	2.5	585 (2)	51900	5700	12	36	11	1

* ( ) = total C level.

** ( ) = extractable P.

3.1.2.2 Immediate effects of amendation. The immediate effects of amendation on some of the physical and chemical characteristics of the three minespoils (Table 6b) were:

i) Grassland - the SAR of the minespoil after the addition of gypsum decreased in comparison with the control; also the level of ammonium acetate extractable Na increased in the gypsum treatment. The SAR and levels of extractable Na were lowest in the topsoil layer placed on the surface of the spoil. The SAR of the spoil immediately below the topsoil (i.e. 15-25 cm) was not affected by topsoil treatment. The pH of the spoil was not altered by amendation.

ii) Subalpine and Oil Sands - fertilizer decreased the pH slightly in the 0 to 5 cm depth in both these spoils. The sewage sludge treatment of the extracted oil sands also resulted in a slight drop in pH, but only at the 5-15 cm depth. As expected, extractable K and Na levels were increased by fertilization.

Tables 7, 8 and 9 present the effects of amendation on DTPA extractable metals shortly after application of the amendments, but prior to planting.

i) Grassland spoil plots - sewage sludge significantly increased the Cu, Zn and Pb levels in the 0-5 cm depth of soil compared with the levels measured in the other treatments. However, sludge-amended soil metal levels were very low in comparison with those found in the sludge prior to its addition to the soil (see Table 6a). The effects of amendation on metal levels in the deeper soil were not significant.

ii) Subalpine minespoil - as in the grassland spoil plots, sewage sludge amendation significantly increased the Cu, Zn and Pb levels over that measured in the control plots, but again only in the 0-5 cm depth of soil. The addition of peat increased the Fe and Zn levels in the surface soil. No significant amendation effects on extractable metal levels were recorded for the subsurface soil.

iii) Extracted oil sands - as for the previous two minespoils sewage sludge increased soil Cu and Zn levels, while peat amendation increased the levels of Fe and Zn in the 0-5 cm soil. However, contrary to the previous two minespoils sewage and peat

Table 6b. Some chemical and physical properties of minespoils after amendation.

Spoil Type	Treatment and Depth (cm)	pH	E.C.^{1} (mS/cm)	Extractable cations^2 (me l^{-1}) Ca	Mg	Na	K	SAR^3
Grassland	Control: 0-5	7.2^{a}	4.7^{b}	4.4^{a}	1.4^{a}	47.0^{b}	0.3^{a}	27.8^{b}
	Gypsum	7.0^{a}	8.8^{c}	21.8^{b}	6.3^{b}	87.4^{c}	0.6^{c}	23.3^{b}
	Sewage Sludge	7.3^{a}	5.1^{b}	5.1^{a}	1.8^{a}	50.9^{b}	0.4^{b}	27.3^{b}
	Topsoil	7.1^{a}	1.5^{a}	6.6^{a}	2.1^{a}	8.5^{a}	0.7^{d}	4.2^{a}
	Control: 5-15	7.5^{b}	4.4^{a}	3.8^{a}	1.2^{a}	42.8^{a}	0.3^{a}	27.4^{a}
	Gypsum	7.1^{a}	8.1^{b}	15.8^{b}	4.8^{b}	79.5^{b}	0.5^{b}	24.8^{a}
	Sewage Sludge	7.5^{b}	5.7^{ab}	5.6^{a}	2.0^{ab}	55.8^{ab}	0.3^{a}	28.8^{a}
	Topsoil: 15-25	7.4^{ab}	5.3^{a}	5.8^{a}	1.8^{ab}	53.9^{ab}	0.4^{a}	29.0^{a}
Subalpine	Control: 0-5	7.1^{b}	0.5^{a}	3.9^{a}	1.1^{a}	1.0^{a}	0.1^{a}	0.45^{a}
	Fertilizer	6.7^{a}	4.2^{b}	29.9^{b}	7.3^{b}	0.9^{a}	2.0^{c}	0.27^{a}
	Sewage Sludge	6.9^{ab}	1.8^{a}	19.4^{b}	6.4^{b}	2.7^{b}	0.6^{b}	0.33^{a}
	Peat	7.0^{ab}	2.3^{ab}	24.9^{b}	8.1^{b}	0.8^{a}	0.3^{b}	0.58^{a}
	Control: 5-15	7.1^{a}	0.5^{a}	1.5^{a}	0.9^{a}	0.7^{a}	0.1^{a}	0.45^{a}
	Fertilizer	7.1^{a}	0.6^{a}	1.7^{a}	1.3^{a}	0.5^{a}	0.1^{a}	0.27^{a}
	Sewage Sludge	7.1^{a}	0.7^{a}	1.8^{a}	1.6^{a}	0.6^{a}	0.1^{a}	0.33^{a}
	Peat: 15-25	7.1^{a}	0.6^{a}	1.6^{a}	1.0^{a}	0.9^{a}	0.1^{a}	0.58^{a}
Oil Sands	Control: 0-5	7.3^{b}	0.5^{a}	3.3^{a}	0.7^{a}	0.8^{a}	0.1^{a}	0.53^{ab}
	Fertilizer	6.4^{a}	3.0^{c}	3.0^{a}	1.5^{a}	1.7^{c}	5.3^{c}	1.00^{b}
	Sewage Sludge	6.8^{ab}	2.0^{b}	8.8^{ab}	4.5^{b}	1.5^{bc}	0.8^{b}	0.60^{ab}
	Peat	7.1^{b}	1.6^{b}	13.4^{b}	5.3^{b}	0.9^{ab}	0.2^{a}	0.29^{a}

(cont'd)

Table 6b. (cont'd).

Spoil Type	Treatment and Depth (cm)	pH	E.C.[1] (mS/cm)	Extractable cations[2] (me l^{-1})				SAR[3]
				Ca	Mg	Na	K	
	Control: 5-15	7.5^c	0.4^a	2.4^a	0.6^a	0.8^a	0.2^a	$.59^a$
	Fertilizer	7.1^b	0.7^a	3.5^a	1.2^{ab}	1.6^b	0.5^{ab}	$.75^a$
	Sewage Sludge	6.4^a	1.8^b	11.6^b	4.6^c	1.5^b	0.6^b	$.53^a$
	Peat: 15-25	7.1^b	1.0^{ab}	7.6^{ab}	3.0^{bc}	0.8^a	0.2^a	$.36^a$

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[1] E.C. - electrical conductivity; [2] Determined by soil paste method; [3] SAR - sodium adsorption ratio.

Data analyzed by one-way ANOVA and Scheffé confidence intervals to locate significant differences. Grassland Mg and (0-5) K, subalpine (0-5) Mg, oil sands (0-5) K data was ln (y + 1) transformed prior to analysis. Grasslands (0-5) SAR, subalpine (0-5) Ca and K and oil sands (5-15) K data analyzed by Kruskal-Wallis test.

Table 7. Effect of amendation on DTPA-extractable metals in grassland minespoil plots prior to planting.

Depth (cm)	Treatment	Metals (µg/g dry soil)				
		Ni	Fe	Cu	Zn	Pb
0-5	Control	1.6^{b}[1]	29.6^{a}	3.2^{b}	6.5^{b}	1.0^{a}
	Gypsum	1.8^{b}	25.6^{a}	3.1^{b}	5.6^{b}	0.9^{a}
	Sewage	1.9^{b}	31.7^{a}	4.5^{c}	8.9^{c}	3.0^{b}
	Topsoil	0.7^{a}	18.5^{a}	1.4^{a}	2.3^{a}	1.3^{a}
5-15 (15-25 in topsoil)	Control	1.5^{a}	29.4^{a}	3.1^{a}	6.3^{a}	0.9^{a}
	Gypsum	1.9^{ab}	38.9^{a}	3.3^{a}	6.6^{a}	0.9^{a}
	Sewage	1.5^{a}	29.0^{a}	3.3^{a}	6.4^{a}	1.3^{a}
	Topsoil	2.3^{b}	48.1^{a}	4.0^{a}	7.6^{a}	0.9^{a}

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[1] Values for each metal in each data set were subjected to a one-way ANOVA. Where the F statistic was significant, Scheffé confidence intervals were computed. Values in each column at each depth followed by the same letter, do not differ significantly ($p = 0.05$).

Table 8. Effect of amendation on DTPA-extractable metals in subalpine minespoil plots prior to planting.

Depth (cm)	Treatment	Metals (μg/g dry soil)				
		Ni	Fe	Cu	Zn	Pb
0-5	Control	0.3^{a}[1]	16.1^{b}	1.6^{ab}	0.9^{a}	0.5^{a}
	Fertilizer	0.6^{a}	2.0^{a}	1.9^{b}	1.3^{a}	0.8
	Sewage	0.5^{a}	18.3^{b}	6.7^{c}	8.1^{b}	6.1^{b}
	Peat	1.1^{a}	234.0^{c}	1.2^{a}	10.3^{b}	0.9^{a}
5-15 (15-25 in peat)	Control	0.3^{ab}	12.4^{a}	1.6^{a}	0.6^{a}	0.7^{a}
	Fertilizer	0.5^{b}	2.7^{a}	2.0^{a}	1.1^{a}	0.9^{a}
	Sewage	0.3^{a}	16.1^{a}	1.8^{a}	0.9^{a}	0.9^{a}
	Peat	0.7^{c}	15.3^{a}	2.9^{a}	1.0^{a}	0.5^{a}

---

[1] Values for each metal in each data set were subjected to a one-way ANOVA. Where the F statistic was significant, Scheffé confidence intervals were computed. Values in each column at each depth followed by the same letter, do not differ significantly ($p = 0.05$). Values not followed by a letter could not be included in the analysis because there was 0 variation amongst the 3 replicates within their treatments.

amendation also increased the amount of Zn measured in the deeper soil. The ease with which the sewage sludge and peat were rototilled into the sand probably accounts for the higher Zn levels in the subsurface soil of these two treatments.

3.1.2.3 Effects of time and amendation on N and P. The effects of time and amendation on extractable P, total N and NO_3-N in the various minesoils are presented in Tables 10 to 18.

i) Grasslands minespoil (Tables 10-12) - in the 0-5 cm soil, gypsum and sewage sludge application resulted in significantly higher levels of extractable P than those measured in the control and topsoil treated plots. Amounts of P in the deeper soil were not significantly affected by amendation. Also time did not influence the levels of P extracted from the soil at the two sampling times (Table 10).

Sewage sludge and topsoil increased the total soil N measured in the surface soil, but not the % N assessed in the subsurface soil. Amendation did not significantly influence soil NO_3-N levels in the surface 0-5 cm deep soil at either sampling time, but after the second growing season (1978) levels of NO_3-N in the deeper soil were greatest in the topsoil treated plots.

ii) Subalpine minespoil (Tables 13-15) - fertilizer and sewage sludge significantly increased P levels in the 0-5 cm soil shortly after application while the P content of the deeper soil did not change. However, after two growing seasons, P levels in the 5-15 cm sewage sludge-treated plots had increased significantly. Peat significantly increased the total N of the subalpine minespoil mainly due to the high levels of NO_3-N in this amendment. Fertilizer and sewage sludge also improved the NO_3-N level, but only in the surface soil. It is interesting to note the significant decrease in NO_3-N in the 0-5 cm soil after two growing seasons.

iii) Extracted oil sands (Tables 16-18) - extractable P levels were highest in the fertilizer and sewage sludge amended plots at both sample times. However, in the fertilized plots, amounts of P

Table 9. Effect of amendation on DTPA-extractable metals in extracted oil sands plots prior to planting.

Depth (cm)	Treatment	Metals (μg/g dry soil)				
		Ni	Fe	Cu	Zn	Pb
0-5	Control	0.2	2.6^{a}	0.0	0.3^{a}	0.6^{a}
	Fertilizer	0.4^{a}[1]	4.3^{ab}	0.2^{a}	0.6^{a}	1.2^{a}
	Sewage	0.2	4.4^{ab}	1.1^{b}	2.6^{b}	2.8^{a}
	Peat	0.9^{a}	35.9^{b}	0.3^{a}	10.7^{b}	2.6^{a}
5-15 (15-25 in peat)	Control	0.2	3.2^{a}	0.1^{a}	0.3^{a}	0.3^{a}
	Fertilizer	0.5^{a}	3.5^{a}	0.1	0.3^{a}	1.1^{a}
	Sewage	0.2	3.4^{a}	0.1^{a}	0.9^{b}	1.3^{a}
	Peat	0.5^{a}	1.9^{a}	0.2	0.9^{b}	1.3^{a}

---

[1] Values for each metal in each data set were subjected to a one-way ANOVA. Where the F statistic was significant, Scheffé confidence intervals were computed. Values in each column at each depth followed by the same letter, do not differ significantly (p = 0.05). Values not followed by a letter could not be included in the analysis because there was 0 variation amongst the 3 replicates within their treatments.

Table 10. Effect of time and amendation on extractable P (μg/g dry soil) in grasslands minespoil.

Soil depth (cm)	Amendment	Sampling date 1977 (prior to planting)	 1978 (fall rye plots)	Amendment mean	Amendment	Time	Inter-action
0-5	Control	1.3	5.5	3.4^{a}[2]	***[1]	NS	NS
	Gypsum	18.1	22.8	20.5^{b}			
	Sewage	7.5	40.1	23.8^{b}			
	Topsoil	3.5	1.8	2.7^{a}			
	Yearly Mean	7.6^{a}	17.6^{a}				
5-15 (15-25 in topsoil)	Control	1.4	4.5	3.0^{b}	*	*	NS
	Gypsum	1.2	18.3	9.8^{b}			
	Sewage	2.8	15.5	9.2^{b}			
	Topsoil	0.4	0.8	0.6^{a}			
	Yearly Mean	1.45^{a}	9.8^{b}				

[1] Each data set was analyzed using a two-way ANOVA. *** = $p<.001$; * = $.01 < p \leq .05$; NS = nonsignificant.

[2] Scheffé confidence intervals were calculated for the amendment and yearly means. Values in the amendment mean column or yearly mean row followed by the same letter do not differ significantly ($p = 0.05$).

Table 11. Effect of time and amendation on total soil N (%) in grasslands minespoil.

Soil depth (cm)	Amendment	Sampling date		Amendment mean	Amendment	Time	Inter-action
		1977 (prior to planting)	1978 (fall rye plots)				
0-5	Control	.11	.13	$.12^{a}$[2]	***[1]	**	NS
	Gypsum	.11	.13	$.12^{a}$			
	Sewage	.14	.20	$.17^{b}$			
	Topsoil	.25	.25	$.25^{c}$			
	Yearly Mean	$.15^{a}$	$.18^{b}$				
5-15 (15-25 in topsoil)	Control	.12	.12	$.12^{a}$	NS	NS	NS
	Gypsum	.13	.14	$.14^{a}$			
	Sewage	.13	.15	$.14^{a}$			
	Topsoil	.13	.14	$.14^{a}$			
	Yearly Mean	$.13^{a}$	$.14^{a}$				

[1] Each data set was subjected to a two-way ANOVA. *** = $p \leq .001$; * = $.001 < p \leq .01$; NS = nonsignificant.

[2] Scheffé confidence intervals were computed for the amendment and yearly means. Values in the amendment mean column or yearly mean row do not differ significantly ($p = 0.05$).

Table 12. Effect of time and amendation on soil NO_3-N (μg/g dry soil) in grasslands minespoil.

Soil depth (cm)	Amendment	Sampling date 1977 (prior to planting)	 1978 (fall rye plots)	Amendment mean	Amendment	Time	Inter-action
0-5	Control	6.4	8.3	7.3^{a2}	NS^1	NS	NS
	Gypsum	15.1	3.8	9.5^a			
	Sewage	25.0	17.4	21.2^a			
	Topsoil	25.2	8.6	16.9^a			
	Yearly Mean	17.9^a	9.5^a				
5-15	Control	11.1	6.3^{ab}	--			*
	Gypsum	8.8^{ab}	3.3^a	--			
	Sewage	36.0^{bc}	9.7^{ab}	--			
	Topsoil	19.0^{bc}	63.7^c	--			
	Yearly Mean	--					

---

[1] Each data set was subjected to a two-way ANOVA after a ln transformation. * = $.01 < p \leq .05$; NS = nonsignificant.

[2] Values followed by the same letter do not differ significantly ($p = 0.05$).

[3] Scheffé confidence intervals were computed for the treatment means. Values followed by the same letter do not differ significantly ($p = 0.05$).

Table 13. Effect of time and amendation on extractable P (μg/g dry soil) in subalpine minespoil.

Soil depth (cm)	Amendment	Sampling date		Amendment mean	Amendment	Time	Interaction
		1977 (prior to planting)	1978 (fall rye plots)				
0-5	Control	3.4^{b}	4.7^{bc}[2]				**[1]
	Fertilizer	50.9^{d}	25.5^{cd}				
	Sewage	42.3^{d}	89.2^{d}				
	Peat	1.5^{ab}	0.3^{a}				
5-15 (15-25 in peat)	Control	2.9	5.0^{a}[2]				*
	Fertilizer	4.9^{a}	10.8^{ab}				
	Sewage	6.7^{a}	65.7^{b}				
	Peat	2.7^{a}	3.4^{a}				

---

[1] Each data set was subjected to a two-way ANOVA after a ln transformation. ** = $.001 < p \leq .01$; * = $.01 < p \leq .05$.

[2] Scheffé confidence intervals were computed for the treatment means. Values followed by the same letter in each data set do not differ significantly ($p = 0.05$).

Table 14. Effect of time and amendation on total soil N (%) in subalpine minespoil.

Soil depth (cm)	Amendment	Sampling date		Amendment mean	Amendment	Time	Inter-action
		1977 (prior to planting)	1978 (fall rye plots)				
0-5	Control	$.09^{a}$	$.09^{a}$[2]				***[1]
	Fertilizer	$.13^{a}$	$.33^{a}$				
	Sewage	$.15^{a}$	$.18^{a}$				
	Peat	1.67^{b}	$.13^{a}$				
5-15 (15-25 in peat)	Control	$.09^{bc}$	$.09^{bc}$[2]				**
	Fertilizer	$.09^{abc}$	$.08^{c}$				
	Sewage	$.09^{abc}$	$.17^{ab}$				
	Peat	$.09^{abc}$	$.18^{a}$				

---

[1] Data for 0-5 cm depth was 2 arcsin $\sqrt{p}$ transformed and subjected to a two-way ANOVA. Data for 5-15 cm depth was ln transformed and analyzed by a two-way ANOVA. *** = $p \leq .001$; ** = $.001 < p < .01$.

[2] Scheffé confidence intervals were computed for the treatment means. Values at each depth followed by the same letter(s) do not differ significantly ($p = 0.05$).

Table 15. Effect of time and amendation on soil NO_3-N (μg/g dry soil) in subalpine minespoil.

Soil depth (cm)	Amendment	Sampling date 1977 (prior to planting)	Sampling date 1978 (fall rye plots)	Amendment mean	Amendment	Time	Interaction
0-5	Control	4.0^{a}[2]	3.0^{a}				***[1]
	Fertilizer	150.4^{d}	4.3^{a}				
	Sewage	25.2^{b}	25.2^{b}				
	Peat	448.9^{d}	96.7^{cd}				
5-15 (15-25 in peat)	Control	2.8	2.9	2.9^{a}[3]	*	NS	NS
	Fertilizer	13.1	2.9	8.0^{ab}			
	Sewage	29.7	22.0	25.9^{b}			
	Peat	5.7	25.6	15.7^{b}			
	Yearly Mean	12.8^{a}	13.4^{a}				

---

[1] Data sets for each depth were subjected to a two-way ANOVA after a ln transformation. *** = $p \leq .001$; * = $.01 < p \leq .05$; NS = nonsignificant.

[2] Scheffé confidence intervals were computed for the treatment means. Values followed by the same letter do not differ significantly ($p = 0.05$).

[3] Scheffé confidence intervals were computed for the amendment and yearly means. Values in the amendment mean column or yearly mean row followed by the same letter do not differ significantly ($p = 0.05$).

Table 16. Effect of time and amendation on extractable P (μg/g dry soil) in extracted oil sands.

Soil depth (cm)	Amendment	Sampling date		Amendment mean	Amendment	Time	Inter-action
		1977 (prior to planting)	1978 (fall rye plots)				
0-5	Control	1.2^{ab}	1.6^{ab}[2]				***[1]
	Fertilizer	52.6^{de}	13.6^{c}				
	Sewage	26.4^{cd}	169.3^{e}				
	Peat	2.7^{b}	0.8^{a}				
5-15 (15-25 in peat)	Control	1.0	1.2	1.1^{a}[4]	***[3]	NS	NS
	Fertilizer	4.7	4.9	4.8^{b}			
	Sewage	23.2	60.3	41.8			
	Peat	1.6	0.9	1.3			
	Yearly Mean	2.4^{a}	2.3^{a}				

---

[1] Data for the 0-5 cm depth was ln transformed and subjected to a two-way ANOVA. *** = $p < .001$; NS = nonsignificant.

[2] Scheffé confidence intervals were computed for the treatment means. Values followed by the same letter do not differ significantly ($p = 0.05$).

[3] The 1977 sewage data was highly variable (7.7, 5.7, 56.1 μg/g dry soil), and inconsistent with the other data in the set. Hence the sewage values were deleted from two-way ANOVA.

[4] Scheffé confidence intervals were computed for amendment and yearly means. Values in the amendment mean column or yearly mean row followed by the same letter do not differ significantly ($p = 0.05$). Sewage data was excluded from these calculations, but it can be safely assumed that the extractable P in the 1978 sewage-amended soil was significantly greater than in the other treatments. The variability in the 1977 sewage data indicates it was not significantly different from the other treatments.

Table 17. Effect of time and amendation on total soil N (%) in extracted oil sands.

Soil depth (cm)	Amendment	Sampling date		Amendment mean	Amendment	Time	Inter- action
		1977 (prior to planting)	1978 (fall rye plots)				
0-5	Control	0	.01	.005^{a}[2]	**[1]	NS	NS
	Fertilizer	.01	.01	.01^{a}			
	Sewage	.02	.05	.04^{a}			
	Peat	.82	.68	.75^{b}			
	Yearly Mean	.21^{a}	.19^{a}				
5-15 (15-25 in peat)	Control	0	.01	.005^{a}[2]	**	NS	NS
	Fertilizer	.01	.01	.01^{a}			
	Sewage	.01	.01	.01^{a}			
	Peat	.06	.07	.065^{b}			
	Yearly Mean	.02^{a}	.025^{a}				

---

[1] Because the 1977 and 1978 control treatments had a mean of 0 with 0 variation and because the 1978 peat treatment at 0-5 cm contained only 1 replicate, the Tukey test for additivity was applied to the averages of each treatment for each depth to test for interactions. Since there were no significant interactions the two-way ANOVA with 1 replication/treatment was applied to each data set at each depth. ** = $.001 < p \leq .01$; NS = nonsignificant.

[2] Scheffé confidence intervals were computed for the amendment and yearly means. Values in the amendment mean column or yearly mean row followed by the same letter are not significantly different ($p = 0.05$).

Table 18. Effect of time and amendation on soil NO_3-N (µg/g dry soil) in extracted oil sands.

Soil depth (cm)	Amendment	Sampling date		Amendment mean	Amendment	Time	Inter- action
		1977 (prior to planting)	1978 (fall rye plots)				
0-5	Control	0.1	0.8^{ab}[2]				**[1]
	Fertilizer	39.9^{cde}	2.7^{ab}				
	Sewage	16.2^{bcd}	7.0^{abc}				
	Peat	251.3^{e}	61.8^{de}				
5-15 (15-25 in peat)	Control	0.1	0.7	0.4^{a}[3]	***	*	NS
	Fertilizer	5.1	1.3	3.2^{a}			
	Sewage	25.5	5.1	15.3^{b}			
	Peat	24.0	8.4	16.2^{b}			
	Yearly Mean	13.7^{a}	3.9^{b}				

---

[1] Data for 0.5 cm depth was ln (x + 1) transformed and subjected to a two-way ANOVA, while the 5-15 cm data was ln transformed prior to two-way ANOVA. *** = $p < .001$, ** = $.001 < p \leq .05$; NS = nonsignificant.

[2] Scheffé confidence intervals were computed for treatment means. Values followed by the same letter do not differ significantly ($p = 0.05$).

[3] Scheffé confidence intervals were computed for the amendment and yearly means. Values within the amendment mean column or yearly mean row followed by the same letter do not differ significantly ($p = 0.05$).

decreased significantly between the two sampling times (presumably due to leaching and plant uptake), while P in the sludge-treated plots increased significantly (probably as a result of the decomposition and subsequent release of P from the sewage sludge aggregates).

As in the subalpine minespoil, peat amendation significantly increased total soil N at both sampling depths. All three amendments significantly increased NO_3-N levels after addition to the sand with the peat amended plots showing the greatest increase. Only sewage sludge and peat increased the NO_3 levels in the subsurface soil. Levels of NO_3 in the fertilized and peat treated soil decreased significantly between the two sampling times.

3.1.2.4 Short term effect of fertilization. The short term (4 mo) effects of fertilizer application on the extractable P, NO_3-N and total N measured at two depths in the subalpine and oil sand minespoils are presented in Table 19. The data show that:

i) the extractable P in the subalpine spoil did not change significantly between the two sampling times whereas there was a significant decrease in P in the fertilized oil sands over the test period. Also the P in the oil sands did not appear to accumulate in the subsurface soil indicating that in a sandy soil such as this P would move rapidly out of the rooting zone.

ii) NO_3-N levels decreased significantly between the sampling times in both spoil types. It did not accumulate in the deeper 5-15 cm soil.

iii) total N had decreased significantly in the subalpine 0-5 cm soil 4 months after fertilization, but no significant decrease occurred in the 0-5 cm depth of the fertilized oil sand. In neither spoil was there any increase in % N in the 5-15 m depth.

between the two sampling times whereas there was a significant decrease in P in the fertilized oil sands over the test period. Also the P in the oil sands did not appear to accumulate in the subsurface soil indicating that in a sandy soil such as this P would move rapidly out of the rooting zone.

Table 19. Changes with time of total N and extractable P and NO_3-N concentrations at two depths in a subalpine minespoil and oil sand tailings amended with mineral fertilizer.

Spoil Type	Sampling Time	Extractable P ($\mu g\ g^{-1}$)		NO_3-N ($\mu g\ g^{-1}$)		N (%)	
		0-5 cm	5-15 cm	0-5 cm	5-15 cm	0-5 cm	5-15 cm
Subalpine[1]	Immediately after fertilizer application	51^a	5^a	150^a	11^a	0.12^a	0.09^a
	Four mo. after application	46^a	20^a	40^b	14^a	0.05^b	0.09^a
Oil Sands[2]	Immediately after fertilizer application	53^a	5^a	38^a	3^a	0.01^a	0.01^a
	Four mo. after application	17^b	7^a	0.5^b	0.5^a	0.01^a	0.01^a

[1] Subalpine data analyzed by a t-test. Values in each column followed by same letter do not differ significantly.

[2] Oil Sands data analyzed by Hotelling's T^2 test. Values in each column followed by same letter do not differ significantly. NO_3-N data required ln (y + 1) transformation.

ii) NO_3-N levels decreased significantly between the sampling times in both spoil types. It did not accumulate in the deeper 5-15 cm soil.

iii) total N had decreased significantly in the subalpine 0-5 cm soil 4 months after fertilization, but no significant decrease occurred in the 0-5 cm depth of the fertilized oil sand. In neither spoil was there any increase in % N in the 5-15 m depth.

3.2 PLANT PRODUCTION (TANK STUDY)

3.2.1. Prairie Grassland Minespoil

The effects of amendation on shoot production of crested wheat, fall rye, russian wild rye and rambler alfalfa over the first three growing seasons are presented in Tables 20 to 23 and are summarized in Fig. 2. The data provided indicate that:

i) shoot production of all test plant species was very low until after the second growing season.

ii) sewage sludge and topsoil promoted the greatest shoot production of crested wheat compared with gypsum and control treatments. Shoot weights increased until the end of the third growing season.

iii) fall rye produced the greatest shoot weight in the sewage sludge and topsoil treated spoil. Shoot weights were highest after the second growing season, but were virtually zero after the third growing season.

iv) growth of russian wild rye was significantly greater in the sewage sludge treated soil; also, like crested wheat, shoot production by this plant species increased over the sampling time.

v) shoot weights measured for rambler alfalfa were highest in the topsoil treatments. Only in the topsoil did shoot production increase with time. It appeared that the alfalfa had difficulty establishing itself in all but the topsoil treated minespoil. Therefore plant distribution was very patchy resulting in extreme variation.

vi) root production of fall rye and alfalfa were measured only after the second growing season. The data obtained are

Table 20. Shoot production (g dwt m^{-2}) for crested wheat grown in amended grassland spoil[1,2].

Amendment	Shoot wt (g dwt m^{-2})			Amendment mean	Significance Level[3]		
	1977	1978	1979		Amendment	Time	Interaction
Control	4.9	167.6	305.9	159.5^a	***	***	NS
Gypsum	12.0	219.2	310.1	180.4^a			
Sewage	47.8	401.6	1399.2	616.2^b			
Topsoil	168.9	360.7	1013.3	514.3^b			
Yearly mean	58.4^a	287.3^b	757.1^b				

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[1] Data was statistically analyzed using nonparametric tests because of significantly heterogenous variances. Values were first analyzed for interaction effects using a test of interactions in replicated factorial design based on alignment. No interaction effects were found and significant effects of time and amendment were determined using Friedman's test for two or more observations / exp. unit.

[2] Values superscripted differently, differ at $p = 0.05$ as indicated by posthoc comparisons based on the Friedman's test.

[3] Levels of significance: *** = $p \leq 0.001$, NS = nonsignificant.

Table 21. Shoot production (g dwt m^{-2}) for fall rye grown in amended grassland spoil[1,2].

Amendment	Shoot wt (g dwt m^{-2})			Amendment mean	Significance Level[3]		
	1977	1978	1979		Amendment	Time	Interaction
Control	62.9	233.4	2.6	99.6^a	***	***	NS
Gypsum	54.3	91.8	0.0	48.7^a			
Sewage	133.8	636.3	31.1	268.1^b			
Topsoil	233.7	857.8	3.5	365.1^{ab}			
Yearly mean	121.2^a	454.8^a	10.2^b				

[1] Data was statistically analyzed using nonparametric tests because of significantly heterogenous variances. Values were first analyzed for interaction effects using a test of interaction in replicated factorial design based on alignment. No interaction effects were found and significant effects of time and amendment were determined using Friedman's test for two or more observations / exp. unit.

[2] Values superscripted differently, differ at $p = 0.05$ as indicated by posthoc comparisons based on the Friedman test.

[3] Levels of significance: *** = $p \leq 0.001$, NS = nonsignificant.

Table 22. Shoot production (g dwt m^{-2}) for russian wild rye grown in amended grassland spoil[1,2].

Amendment	Shoot wt (g dwt m^{-2})			Amendment	Significance Level[3]		
	1977	1978	1979	mean	Amendment	Time	Interaction
Control	7.7	179.8	290.3	159.3^a	***	***	NS
Gypsum	7.3	50.6	501.3	186.4^a			
Sewage	15.2	323.7	1049.7	462.9^c			
Topsoil	26.8	91.6	468.5	195.6^{bc}			
Yearly mean	14.3^a	161.4^b	577.5^c				

[1] Values superscripted differently, differ at $p = 0.05$ based on a two-way ANOVA and Scheffé confidence intervals. Significant differences between means were obtained after ln $(x + 1)$ transformation.

[2] Levels of significance: *** = $p \leq 0.001$, NS = nonsignificant.

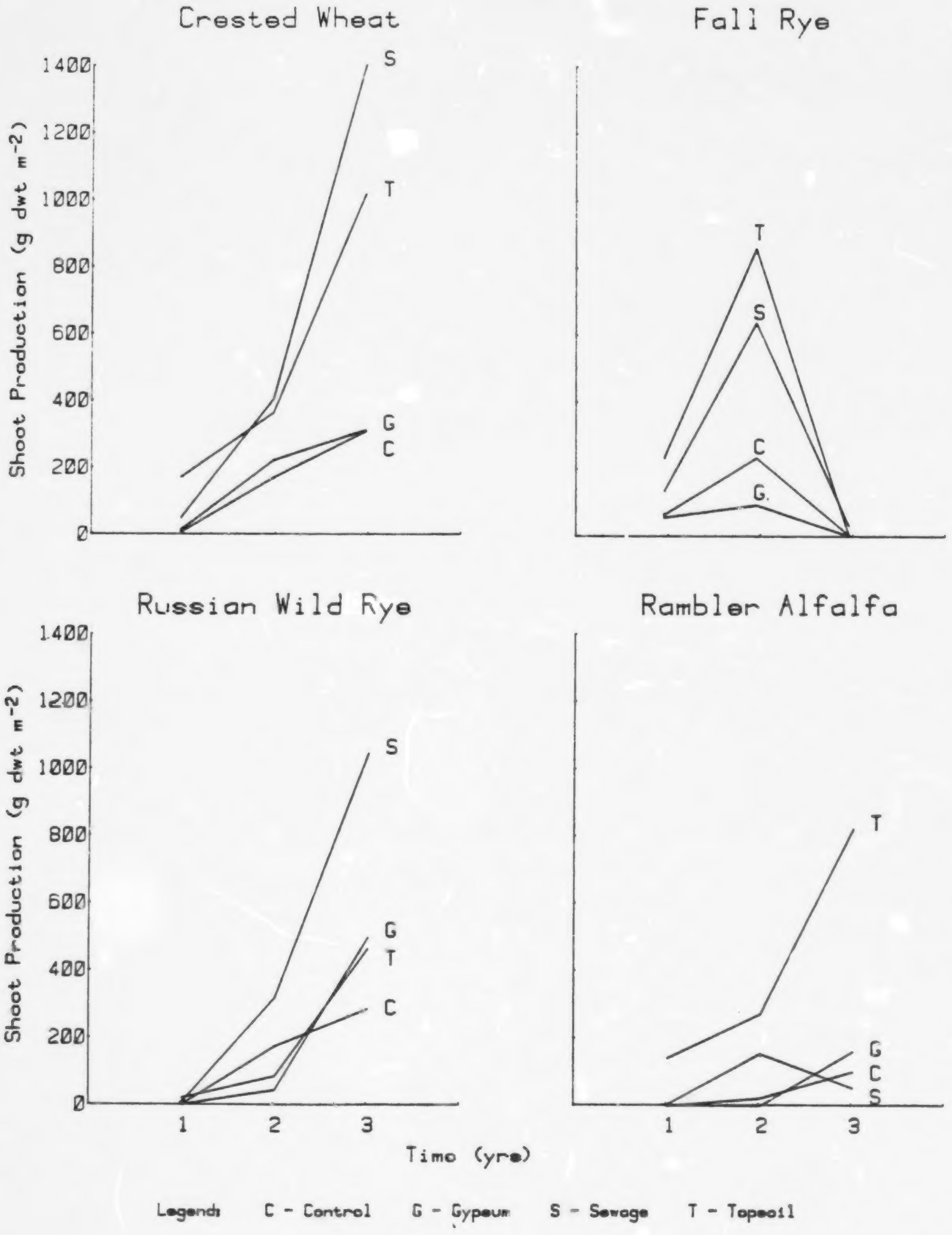
Table 23. Shoot production (g dwt m^{-2}) by rambler alfalfa grown in amended grassland spoil[1,2].

	Shoot wt (g dwt m^{-2})			Amendment	Significance Level[3]		
Amendment	1977	1978	1979	mean	Amendment	Time	Interaction
Control	0.6	22.7	101.8	41.7^a	***	NS	NS
Gypsum	1.3	0	161.5	54.3^a			
Sewage	4.2	155.6	53.6	71.1^a			
Topsoil	141.4	272.9	823.2	412.5^b			

[1] All values were transformed [ln (x + 1)] and subjected to a two-way ANOVA. *** = $p < .001$, NS = nonsignificant.

[2] Scheffé confidence intervals were computed for the amendment means. Values in the amendment mean column followed by the same letter do not differ significantly ($p = .05$).

Fig. 2. Shoot production in amended grassland spoil.



presented in Table 24. Generally the root weights mirrored the shoot weights, with sewage sludge and topsoil stimulating the greatest root production of fall rye and topsoil promoting the best root growth of rambler alfalfa.

3.2.2 Subalpine Minespoil

Shoot and root production of slender wheatgrass, alsike clover, white spruce and willow in the variously amended subalpine minespoil plots are presented in Tables 25 to 29. Shoot production results are summarized in Fig. 3. These data show that:

i) shoot production of slender wheatgrass (Table 25) was greatest in the sewage sludge and fertilizer amended spoil until the third growing season, when slender wheatgrass sown in the peat amended spoil demonstrated the most growth. As for the fall rye grown in the grassland spoil, shoot production of slender wheatgrass peaked after the second growing season and then decreased dramatically (especially in the sludge and fertilizer-treated spoil). During the third growing season, shoot production did not vary significantly amongst the three amendment treatments.

ii) unlike slender wheatgrass, shoot production by alsike clover (Table 26) increased significantly in all treatments over the three growing seasons. Fertilizer and sewage sludge amendation resulted in the best growth response. Although no data were collected on the reseeding success of the grass and legume species, field observations indicated that reseeding success of alsike clover was higher than that of slender wheatgrass and the two legumes tested in the grassland and oil sands minespoil.

iii) root production of slender wheatgrass (Table 27) measured after two growing seasons was significantly greater in the amended spoil compared with the control spoil. On the other hand, alsike clover root production did not appear to be affected by amendation and tended to be less than that measured for slender wheatgrass. This may have been a result of the sampling technique employed. Soil for root production estimates was sampled to a depth of 14 cm (due to the rocky nature of the subalpine spoil material, it was virtually

Table 24. Effect of amendation of prairie grassland spoil on root production over two growing seasons.

	Root wt (g dry root m^{2-1})			
Plant Species	Control	Gypsum	Sewage Sludge	Topsoil
Fall Rye	53.4a[1]	63.2^{a}	264.9^{b}	457.7^{c}
Rambler Alfalfa	52.1^{a}[2]	6.5^{a}	17.9^{a}	256.6^{a}

[1] The nonparametric Friedman test with posthoc comparisons at $p = 0.05$ was used to determine significant effects of amendation on root wt.

[2] A one-way ANOVA after a $\ln(y + 1)$ transformation was applied to alfalfa data. Values in each row followed by the same letter do not differ significantly ($p = 0.05$).

Table 25. Shoot production (g dwt m^{-2}) for slender wheatgrass grown in amended subalpine spoil[1].

Amendment	Shoot wt (g dwt m^{-2})			Significance Level[2]		
	1977	1978	1979	Amendment	Time	Interaction
Control	2.6^{a}	43.1^{cd}	21.0^{bc}	***	***	***
Peat	6.9^{ab}	111.1^{cde}	98.2^{cde}			
Fertilizer	30.0^{bc}	209.5^{de}	44.7^{cd}			
Sewage	53.2^{cd}	324.6^{e}	63.7^{cd}			

[1] Data analysed by 2-way ANOVA after ln Y transformation. Numbers followed by the same letter are not significantly different (p = .01) as determined by Scheffé multiple contrasts.

[2] *** = p < .001.

Table 26. Shoot production (g dwt m^{-2}) for alsike clover grown in amended subalpine spoil.

Amendment	1977	1978	1979	Amendment mean	Amendment	Time	Interaction
Control	2.9	42.2	202.3	82.5^{a}[2]	***[1]	***	NS
Fertilizer	40.0	231.5	355.7	209.1^{b}			
Sewage	11.2	131.2	371.9	171.4^{ab}			
Peat	0.7	53.1	166.1	73.3^{a}			
Yearly Mean	13.7^{a}	114.5^{b}	274.0^{c}				

[1] All values were transformed [ln (x + 1)] and subjected to a two-way ANOVA. *** = $p < .001$, NS = not significant.

[2] Scheffé confidence intervals were computed for the yearly and amendment means. Values in the amendment mean column or the yearly mean row followed by the same letter do not differ significantly ($p = .05$).

Table 27. Root production in amended subalpine minespoil after two growing seasons.

Plant Species	Root wt (g dry root m^{2-1})			
	Control	Fertilizer	Sewage Sludge	Peat
Slender Wheatgrass	36.0a[1]	141.5^b	249.5^b	139.4^b
Alsike Clover	78.2^a[2]	21.1^a	126.8^a	43.5^a

[1] The nonparametric Friedman test with posthoc comparisons at $p = 0.05$ was used to analyze slender wheatgrass data.

[2] A one-way ANOVA was applied to the clover data. Values in each row followed by same letter do not differ significantly ($p = 0.05$).

Table 28. Effect of amendation on the growth of white spruce planted in subalpine minespoil.

Amendment	Shoot wt. (g)	Root wt. (g)	Height (cm)
	Growth after one growing season		
Preplanting	.17a[1]	.09^{a}	5.6^{a}
Control	.29^{a}	.37^{b}	6.3^{a}
Fertilizer	.36^{a}	.31^{b}	6.1^{a}
Sewage	.42^{a}	.35^{b}	6.3^{a}
Peat	.32^{a}	.30^{b}	5.4^{a}
	Growth after two growing seasons		
Control	1.54^{a}	.88^{a}	11.0^{a}
Fertilizer	1.72^{a}	1.08^{a}	10.8^{a}
Sewage	2.26^{a}	1.31^{a}	11.6^{a}
Peat	1.27^{a}	.79^{a}	9.3^{a}
	Growth after three growing seasons		
Control	6.13^{a}		16.2^{a}
Fertilizer	5.64^{a}		13.6^{a}
Sewage	9.38^{a}		17.7^{a}
Peat	6.95^{a}		16.6^{a}
	Growth after four growing seasons		
Control	13.8^{ab}		22^{a}
Fertilizer	10.8^{a}		20^{a}
Sewage	21.2^{b}		24^{a}
Peat	21.4^{b}		26^{a}

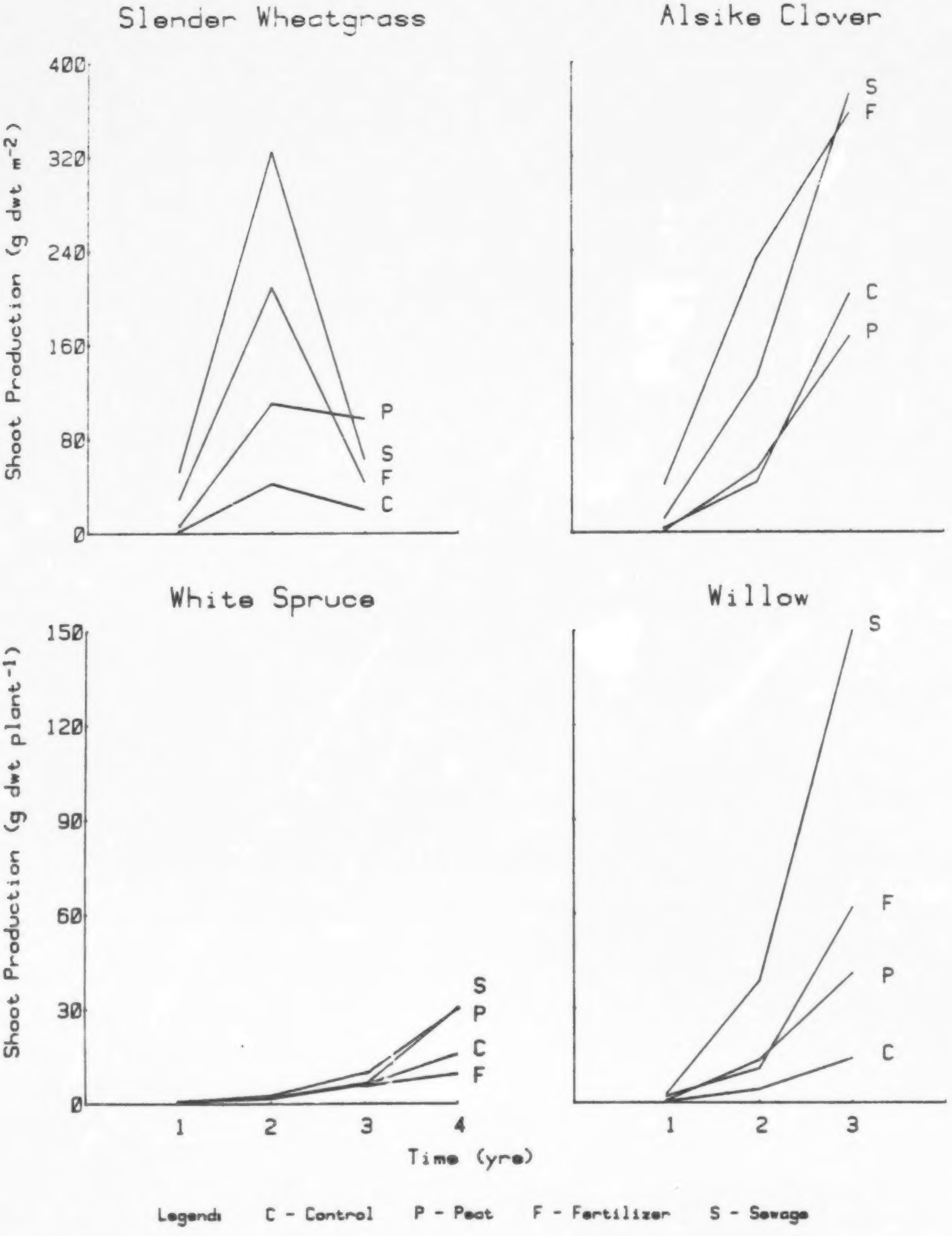
[1] Data was analyzed by 1-way ANOVA and Scheffé confidence intervals. Values in each column in each growing season followed by the same letter do not differ significantly ($p = 0.05$). Shoot data for first and third growing seasons required a log transformation, while first growing season root data required a square root transformation.

Table 29. Effect of amendation on the growth of willow in subalpine minespoil.

Amendment	Shoot wt. (g)	Root wt. (g)	Height (cm)
	Growth after one growing season		
Preplanting	.30a[1]	$.10^{a}$	15.9^{a}
Control	$.65^{a}$	$.65^{b}$	15.9^{a}
Fertilizer	2.30^{c}	1.46^{c}	25.8^{b}
Sewage	3.00^{c}	1.74^{c}	31.3^{ab}
Peat	$.91^{b}$	$.69^{b}$	16.7^{a}
	Growth after two growing seasons		
Control	4.55^{a}		31.7^{b}
Fertilizer	11.06^{b}		51.3^{b}
Sewage	38.78^{c}		50.1^{b}
Peat	13.73^{b}		72.2^{c}
	Growth after three growing seasons		
Control	14.40^{a}		40.1^{a}
Fertilizer	62.03^{ab}		69.7^{a}
Sewage	151.77^{c}		134.1^{b}
Peat	41.13^{b}		68.1^{a}

[1] Data was analyzed by 1-way ANOVA and Scheffé confidence intervals. Values in each column for each growing season followed by the same letter do not differ significantly ($\alpha = 0.05$). Height data for first two growing seasons required natural log transformation prior to analysis.

Fig. 3. Shoot production in amended sub-alpine spoil.



impossible to sample any deeper). Clover seemed to root deeper than slender wheatgrass, hence it is likely that clover root estimates would have been more comparable to slender wheatgrass root estimates if deeper samples had been processed. Also, amendment effects on clover root growth may have been found to be more pronounced if deeper samples had been taken.

iv) amendation effects on shoot production of white spruce (Table 28) were not significant until the fourth growing season, when shoot weights measured in the sewage and peat-treated plots were greater than those in the control and fertilizer treatments. The absence of significant amendation effects prior to the fourth growing season was presumably due to the slow growth of white spruce.

Root weights during the first growing season increased in all the test plots over that measured at pre-planting indicating that root growth occurred in all plots after planting (Table 28). However, no significant amendation effects were measured after either one or two growing seasons. Slow growth resulting in slow response to nutrients, and high variation may explain these results.

Height of white spruce was not affected by treatment at any of the sampling times indicating that a height measurement is not an accurate method for assessing growth response (Table 28). For example, height of white spruce after four growing seasons was not significantly different amongst any of the treatments although shoot weights in sewage sludge and peat-amended plots were double those measured in control and fertilized plots.

v) shoot production of willow (Table 29) was more stimulated by sewage sludge than by any other amendment. This was true after all three growing seasons. Growth in the fertilizer-treated soil was highly variable.

Root weights measured after the first growing season demonstrated that both sewage sludge and fertilizer were most effective in promoting root growth (Table 29). These data correlated with that obtained for shoot production.

Willow had the greatest shoot height in the fertilized minespoil after one growing season, in the peat-amended soil after the second growing season and in the sewage sludge treated soil after the third growing season (Table 29). Again the height data is misleading because dieback of the willow shoots was significant in the peat treatment after the second growing season (Table 30) and grazing of willow by deer during the winter months was sometimes excessive.

vi) the effect of amendment type on survival of spruce and willow was measured after the first and second growing seasons (Table 30). Survival was high for both willow and spruce and was not significantly influenced by treatment. However, dieback of willow was significant in the peat-treated minespoil.

3.2.3. Oil Sands Minespoil

Data on growth of slender wheatgrass, sainfoin, jack pine and bearberry in the variously amended oil sand tailings are presented in Tables 31 to 36 and shoot production is summarized in Figure 4. These data show that:

i) estimates of shoot production of slender wheatgrass (Table 31) were highly variable making it difficult to detect any significant amendment effects on plant growth. Nevertheless, shoot production tended to be highest after the second growing season, with sewage sludge promoting the most growth followed by fertilizer and peat. It is interesting that the pattern of grass growth in the variously amended oil sand spoil was very similar to that obtained for slender wheatgrass in the subalpine spoil (i.e. a peak in plant production after the second growing season followed by very little plant growth in the third growing season, particularly in the sewage sludge and fertilizer treated sand). Very little reseeding by slender wheatgrass was observed to occur in the grass subplots, although seed germinated quite readily in the unplanted pathways.

ii) shoot weights of sainfoin (Table 32) were also highest after the second growing season. Again sewage sludge was most effective in stimulating shoot growth. Unlike slender wheatgrass, shoot weights remained relatively stable after the second growing season.

Table 30. Survival of white spruce and survival and dieback of willow grown in subalpine minespoil.

Amendment	Percent Survival				Percent dieback (willow)
	Spruce		Willow		
	1 yr	2 yr	1 yr	2 yr	
Control	93a[1]	90a	95a	91a	7a
Fertilizer	93a	89a	97a	94a	12a
Sewage Sludge	89a	84a	92a	91a	8a
Peat	92a	88a	95a	92a	60b

[1] Data analyzed by one-way ANOVA after a 2 arcsin $\sqrt{p}$ transformation. Where applicable Tukey multiple comparisons were used to find differences. Values in each column followed by same letter do not differ significantly ($p = 0.05$).

Table 31. Shoot production (g dwt m^{-2}) for slender wheatgrass grown in amended oil sands spoil.

	Shoot wt (g dwt m^{-2})			Significance Level[2]		
Amendment	1977	1978	1979	Amendment	Time	Interaction
Control	1.6^{a}[1]	1.8^{a}	0.2^{a}			***
Fertilizer	45.8^{ab}	166.6^{bc}	17.7^{a}			
Sewage	101.7^{abc}	766.3^{bc}	49.6^{ab}			
Peat	7.6^{a}	109.6^{abc}	80.4^{ab}			

[1] Data was analyzed by a two-way ANOVA and Scheffé confidence intervals. Values followed by the same letter(s) do not differ significantly.

[2] *** = $p \leq .001$.

Table 32. Shoot production (g dwt m^{-2}) for sainfoin grown in oil sands spoil.

Amendment	1977	1978	1979	Amendment mean	Amendment	Time	Interaction
Control	6.3	65.5	18.1	30.0^{a}[2]	*[1]	*	NS
Fertilizer	106.3	41.1	91.9	79.8			
Sewage	122.8	313.5	314.9	250.4^{b}			
Peat	46.6	219.4	190.3	152.1^{ab}			
Yearly Mean	58.6^{a}	199.5^{b}	174.4^{ab}				

[1] This data could not be transformed and a non-parametric test for an interaction in a two-way design demonstrated a significant interaction. By deleting the fertiizer data, the significant interaction was removed so a Friedman's analysis could be applied to the control, sewage and peat sainfoin data. The results of this test are shown in the above table. * = $0.01 < p \leq 0.05$; NS = non-significant.

[2] Non-parametric posthoc confidence intervals for pairwise comparisons were computed for the yearly and amendment means. Values in the amendment mean column or the yearly mean row followed by the same letter do not differ significantly ($p = .05$).

Table 33. Root production in amended oil sand minespoil after two growing seasons.

	Root wt (g dwt m^{2-1})			
Plant Species	Control	Fertilizer	Sewage Sludge	Peat
Slender Wheatgrass	9^{a1}	181.2^{b}	268.5^{b}	342.2^{b}
Sainfoin	45.2^{a}	24.4^{a}	28.7^{a}	79.6^{a}

[1] Data analyzed by a one-way ANOVA after square root transformation. Values in each row followed by same letter do not differ significantly as determined by Scheffé confidence intervals (p = 0.05).

Table 34. Effect of amendation on the growth of jack pine in oil sands spoil.

Amendment	Shoot wt. (g dwt/plant)	Root wt. (g dwt/plant)	Height (cm)
	Growth after one growing season		
Preplanting	.46^{a}[1]	.20^{a}	11.2^{a}
Control	.49^{a}	.42^{ab}	11.1^{c}
Fertilizer	.99^{ab}	.66^{bc}	10.7^{a}
Sewage	.99^{b}	.86^{c}	10.7^{a}
Peat	1.07^{b}	.72^{bc}	11.4^{a}
	Growth after two growing seasons		
Control	.64^{a}	.52^{a}	14.1^{a}
Fertilizer	2.60^{b}	1.26^{b}	13.4^{a}
Sewage	10.41^{c}	4.42^{c}	17.4^{a}
Peat	2.64^{b}	1.31^{b}	14.1^{a}
	Growth after three growing seasons		
Control	.65^{a}		10.6^{a}
Fertilizer	2.40^{b}		11.6^{a}
Sewage	24.47^{d}		27.9^{b}
Peat	7.71^{c}		15.8^{a}

---

[1] All analysis was done by one-way ANOVA. Posthoc multiple comparisons were used to find differences at $p = 0.05$. Values in each column for each season followed by the same letter do not differ significantly. Shoot and root data required a natural log transformation.

Table 35. Effect of amendation on growth of bearberry planted in oil sands minespoil.

Amendment	Shoot wt. (g)	Root wt. (g)
	Growth after one growing season	
Preplanting	0.99^{ab}[1]	0.21^{a}
Control	0.81^{a}	0.38^{ab}
Fertilizer	1.23^{ab}	0.41^{ab}
Sewage	1.30^{ab}	0.45^{ab}
Peat	1.41^{b}	0.51^{b}
	Growth after two growing seasons	
Control	0.79^{a}	0.58^{a}
Fertilizer	1.16^{a}	0.70^{a}
Sewage	7.58^{b}	1.29^{a}
Peat	5.88^{b}	1.08^{a}

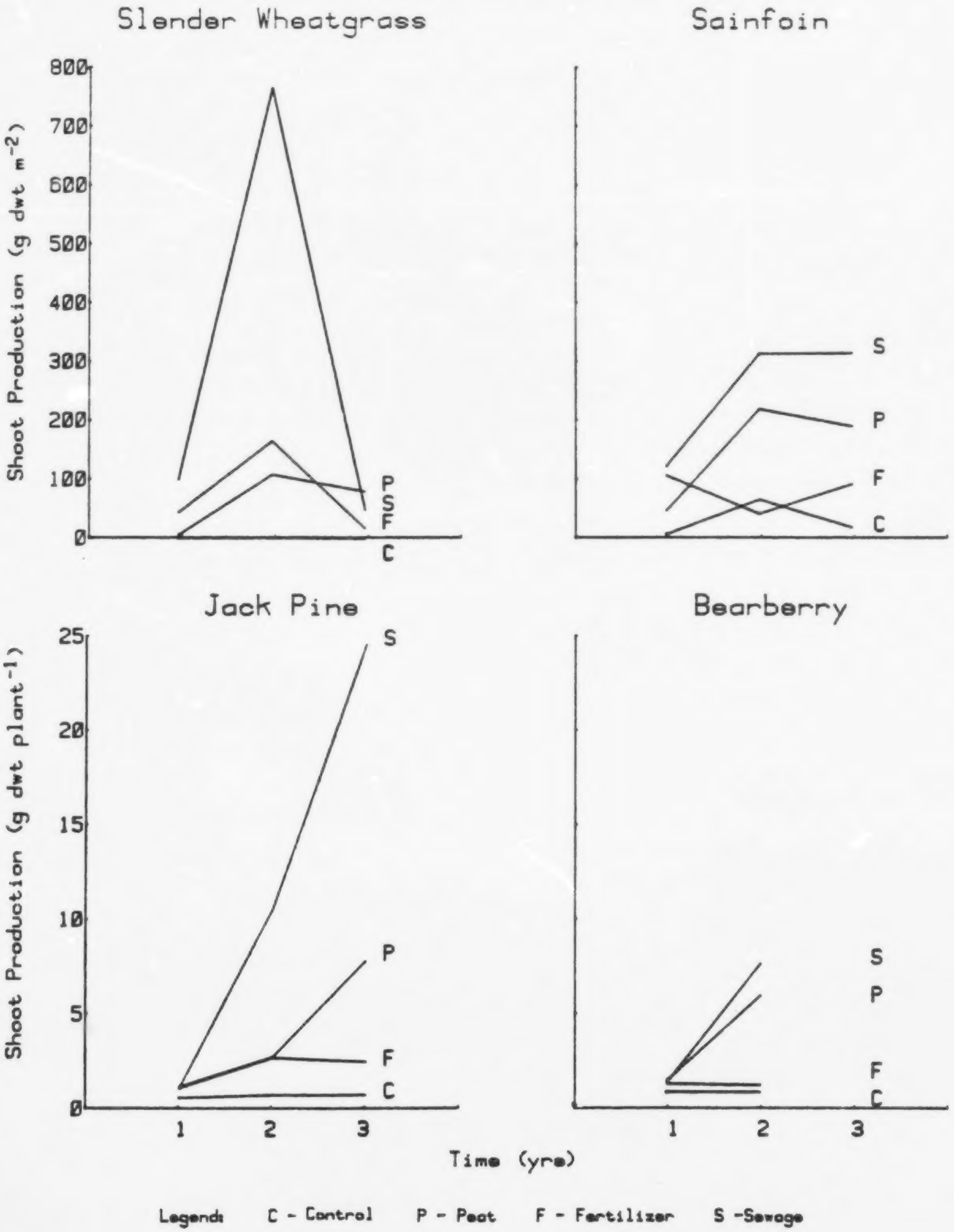
[1] Data analyzed by one-way ANOVA and Scheffé confidence intervals. Values in each column for each season followed by same letter do not differ significantly ($p = 0.05$). Shoot wts. required natural log transformations prior to analysis.

Table 36. Survival of jack pine and survival and dieback of bearberry grown in oil sand.

	Percent Survival				Percent dieback of bearberry
	Jack pine		Bearberry		
Amendment	1 yr	2 yr	1 yr	2 yr	
Control	86^{a1}	54^{a}	62^{a}	40^{ab}	20^{a}
Fertilizer	86^{ab}	80^{ab}	31^{b}	21^{a}	19^{a}
Sewage Sludge	98^{ab}	97^{b}	38^{b}	30^{ab}	1^{a}
Peat	92^{b}	88^{ab}	74^{a}	58^{b}	17^{a}

[1] Data was subjected to a one-way ANOVA and Tukey multiple comparisons. All but the percent dieback data was 2 arcsin $\sqrt{p}$ transformed prior to analysis. Values in each column followed by same letter do not differ significantly ($p = 0.05$).

Fig. 4. Shoot production in extracted Oil Sands.



iii) root production of slender wheatgrass (Table 33) after the second growing season was not significantly different amongst the fertilizer, sewage sludge and peat treatments, indicating that shoot/root ratios were highest in the sewage sludge amended sand. Sainfoin root production like that of alsike clover in the subalpine spoil was not affected by amendment. Again the sampling technique employed may have affected these results, since sainfoin appeared to be a very deep rooting species, particularly in the nutrient-deficient oil sand tailings used in this study.

iv) jack pine (Table 34) were most productive in the sewage sludge treated sand particularly after the second and third growing seasons. Peat was the next best amendment for promoting shoot growth followed by fertilizer. No growth occurred in the untreated sand as a result of the lack of essential nutrients required for plant production. Root growth occurred in all but the control treatments during the first growing season. After the second growing season jack pine roots sampled from the sewage sludge treatment were the heaviest - a result which mirrors the shoot weights obtained for the sludge treatment. Tree height was not influenced by amendation until the third growing season when sewage sludge treated seedlings were significantly taller than those in the other treatments. Deer, grazing on the seedlings during the winter, may have affected the height measurements.

v) bearberry (Table 35) produced the greatest shoot weights in the sewage sludge and peat treated sand particularly after the second growing season. Although root growth was best in the peat-amended spoil during the first growing season, no significant treatment effects on root production were measured after the second growing season.

vi) the survival of jack pine and bearberry after the first and second growing season are presented in Table 36. Jack pine planted in the sewage sludge amended sand demonstrated the best survival. Mortality of pine seedlings in the control treatment was severe between the first and second year after planting. Bearberry demonstrated the poorest survival record of all woody plants investigated in this study. After the first winter, bearberry in the peat and control plots demonstrated better survival than bearberry planted in the sewage sludge-treated and fertilized plots. However, seedling losses in all treatments were high between the first and second year after planting resulting in no significant amendment effects on survival of two-year-old seedlings. The per cent dieback of bearberry was not significantly influened by treatment.

3.3 SOIL FERTILITY AND PLANT GROWTH

In an attempt to determine whether or not shoot production was related to soil P and N status, simple correlation coefficients were calculated for the following:

i) extractable soil P, NO_3-N and total N, measured in soil samples removed from the plots after amendation but prior to planting, and shoot weights of slender wheatgrass (subalpine and oil sands minespoil), alsike clover (subalpine spoil) and sainfoin (oil sands tailings) after the first growing season.

ii) extractable P, NO_3-N and total N assessed for soil samples removed from slender wheatgrass subplots at the conclusion of the second growing season, and shoot production of slender wheatgrass, alsike clover and sainfoin after the second growing season.

The correlation coefficients obtained are presented in Table 37. Generally, extractable P demonstrated the best relationship with shoot weights of slender wheatgrass and first growing season shoot weights of alsike clover. No strong relationships were observed between shoot weights of both grass and legume species and NO_3-N or total soil N. Small sample size (i.e. 12 values per plant species for each parameter tested) and the fact that data from all the treatments were combined into the same correlation matrix could account for the lack of relationship between plant growth and soil P and N status. Also soil samples for nutrient analysis were not removed from the same areas as those clipped for primary production estimates.

Table 37. Correlation coefficients between grass and legume shoot production (g/m^2) and selected soil nutrient levels.

Spoil Type	Plant Species	Growing Season	Correlation Coefficients		
			P	NO_3-N	Total N
Subalpine	Slender Wheatgrass	1	.735	-.366	-.488
		2	.833	-.122	.289
	Alsike Clover	1	.823	-.078	-.353
		2	.295	-.420	.515
Oil Sands	Slender Wheatgrass	1	.434	-.356	-.334
		2	.910	-.214	-.060
	Sainfoin	1	.675	-.185	-.268
		2	.370	.096	.458

3.4 CHEMICAL ANALYSIS OF THE FOLIAGE OF SELECTED PLANT SPECIES

3.4.1. Subalpine Minespoil

Data on the effects of the various amendments on the elemental concentrations in the foliage of slender wheatgrass and needles of white spruce after one growing season are presented in Tables 38 and 39. These data show that:

i) slender wheatgrass - phosphorus was significantly greater in grass grown in the sewage sludge treated spoil, while Ca was significantly concentrated by grasses sampled from the control and peat amended plots. Levels of heavy metals (i.e. Cu, Zn, Fe) were not significantly greater in plants from the sewage sludge treatment than in those from the other treatments.

ii) white spruce - as a result of the slow growth rate of white spruce and hence its slow uptake of essential nutrients, there were no significant amendment effects on nutrient levels assessed after one growing season.

iii) total N (Table 40) measured in the foliage of slender wheatgrass, alsike clover and willow (subalpine minespoil) demonstrated that peat provided the most N for growth in comparison with the other amendments. Interestingly, treatment had no significant effect on N concentrations in the alsike clover, presumably because it can provide its own N through N fixation.

3.4.2 Oil Sand Minespoil

The elemental concentrations of slender wheatgrass, sainfoin and jack pine grown in the variously amended oil sands plots are presented in Tables 41 to 43. These data indicate that:

i) levels of P and K in slender wheatgrass plants from the amended spoil (Table 41) were significantly greater than levels measured in plants from control spoil. Phosphorus was concentrated particularly in grass grown in sewage amended sand. Zinc concentrations were significantly higher in grass from the peat plots while plants sampled from the control plots demonstrated higher Fe levels than plants in the amended plots. Amendment effects on nutrient levels in foliage of slender wheatgrass planted in the

Table 38. Elemental concentrations in foliage of slender wheatgrass after one growing season in amended subalpine spoil.

	Elements ($\mu g\ g^{-1}$)							
Treatment	P	K	Ca	Mg	Mn	Cu	Zn	Fe
Control	2140^a	14500^a	12500^b	2533^a	225^a	4^a	42^a	875
Fertilizer	3577^a	18558^a	4894^a	2496^a	400^a	27^a	40^a	8713^b
Sewage Sludge	5429^b	23772^a	5903^a	2441^a	152^a	36^a	60^a	1233^a
Peat	2757^a	10921^a	10690^b	2097^a	114^a	14^a	98^a	574
df	4	4	4			4	4	
T	4.43	1.11	2.49		1.11	1.47		
95% C.I.	1917	14266	1860		41	62.5		

Only one value was available for the control and peat treatments. Fertilizer and sewage data analyzed by Student t-test. Pooled sample variances were used to construct 95% confidence intervals; control or peat values outside the interval were considered different. df - degrees of freedom, T-t value; C.I. - confidence interval. Mg, Mn, and Fe data analyzed by a Wilcoxan test. Control and peat Fe values are probably different from fertilizer and sludge values. Values in each column followed by same letter do not differ significantly.

Table 39. Elemental concentrations in white spruce needles after one growing season in amended subalpine spoil.

Treatment	Elements ($\mu g\ g^{-1}$)							
	P	K	Ca	Mg	Mn	Cu	Zn	Fe[1]
Control	2778^b	5627^a	12295^a	2175^a	2015^a	9^b	48^a	423^b
Fertilizer	2207^a	5130^a	13218^a	2462^a	1600^a	7^b	40^a	546^b
Sewage Sludge	2562^{ab}	5102^a	12575^a	2549^a	1966^a	8^b	50^a	574^b
Peat	2085^a	4765^a	13314^a	2374^a	1541^a	2^a	41^a	116^a
df	8	8	8	8	8	8	8	8
MSE	34977	424695	2876193	70386	91198	4.6	45	.04

Data analyzed by one-way ANOVA. Significant differences located by Scheffé multiple contrasts. Fe data were ln Y transformed; values are geometric means. Values in each column followed by same letter are not significantly different ($p = 0.05$). df - degrees of freedom; MSE - mean square error.

Table 40. Total N (%) measured in the foliage of selected plant species after 2 growing seasons in amended subalpine spoil.

Treatment	% N		
	Slender wheatgrass	Alsike clover	Willow
Control	1.3^{a}	2.4^{a}	1.9^{a}
Fertilizer	1.0^{a}	2.0^{a}	2.1^{ab}
Sewage Sludge	1.6^{ab}	2.9^{a}	2.7^{ab}
Peat	2.3^{b}	3.5^{a}	3.1^{b}
df	8	8	8
MSE	.07	.44	.14

Data analyzed by one-way ANOVA. Scheffé multiple contrasts used to locate significant differences. Values in each column followed by same letter are not significantly different ($p = 0.05$). df - degrees of freedom; MSE - mean square error.

Table 41. Elemental concentrations of foliage in slender wheatgrass after one growing season in amended oil sands.

	Elements ($\mu g\ g^{-1}$)							
Treatment	P	K	Ca	Mg	Mn	Cu	Zn	Fe
Control	1235^{a}	9384^{a}	20000^{c}	2432^{b}	140^{c}	10^{ab}	90^{bc}	550^{b}
Fertilizer	3664^{b}	19554^{b}	3352^{a}	1016^{a}	92^{b}	16^{ab}	44^{a}	336^{a}
Sewage Sludge	5818^{c}	20645^{b}	3836^{ab}	2115^{b}	59^{a}	33^{b}	78^{b}	256^{a}
Peat	2637^{b}	20432^{b}	7664^{b}	1463^{a}	68^{a}	0^{a}	100^{c}	279^{a}
df	6	6	6	6	6	6	6	6
MSE	175414	748914	1180502	31046	43	T=2.38	62	3379
95% C.I.						27		

Due to insufficient sample only one value was available for the control treatment elements. A one-way ANOVA and Scheffé multiple contrasts were applied to the fertilizer, sewage and peat data. The Scheffé multiple contrast was also used to determine if the control was significantly different from the other treatments. Data in each column followed by same letter are not significantly different ($p = 0.05$). df - degrees of freedom; MSE - mean square error.

Table 42. Elemental concentrations in foliage of sainfoin after one growing season in amended oil sands.

Treatment	Elements ($\mu g\ g^{-1}$)							
	P	K	Ca	Mg	Mn	Cu	Zn	Fe
Control	1452^{a}	7885^{a}	15667^{a}	4864^{a}	245^{c}	13^{a}	70^{a}	222^{ab}
Fertilizer	3158^{bc}	13687^{b}	12294^{a}	4033^{a}	217^{c}	13^{a}	39^{a}	267^{b}
Sewage Sludge	4077^{c}	12325^{ab}	13186^{a}	5092^{a}	160^{b}	13^{a}	64^{a}	211^{ab}
Peat	1585^{ab}	10890^{b}	12310^{a}	4102^{a}	61^{a}	24^{a}	49^{a}	166^{a}
df	6	6	6	6	6	6	6	6
MSE	420233	3460092	249926	197863	365	34	171	526

Due to insufficient plant material, only one value was available for the control treatment for each of the elements. Fertilizer, sewage and peat data analyzed by a one-way ANOVA and Scheffé multiple contrasts. Scheffé multiple contrast value was also used to determine if the control values were different from the other treatments. Values in each column followed by same letter do not differ significantly ($p = 0.05$).

Table 43. Elemental concentrations in foliage of jack pine after one growing season in amended oil sands.

Treatment	Elements ($\mu g\ g^{-1}$)						
	P	K	Ca	Mg	Mn	Zn	Fe
Control	1640^{ab}	6010^{a}	3966^{a}	1451^{b}	528^{c}	66^{b}	176^{b}
Fertilizer	2034^{c}	7065^{a}	2681^{a}	9[illegible]2^{a}	299^{ab}	37^{a}	114^{ab}
Sewage Sludge	1985^{bc}	6129^{a}	3557^{bc}	1204^{ab}	350^{b}	70^{b}	116^{ab}
Peat	1485^{a}	6021^{a}	6291^{d}	1498^{b}	265^{a}	59^{b}	107^{a}
df	8	8	8	8	8	8	8
MSE	18863	193892	161352	25073	.0083	31	573

Data analyzed by a one-way ANOVA. Scheffé multiple contrasts were applied to locate significant differences. Values in each column followed by the same letter do not differ significantly ($p = 0.05$). df = degrees of freedom; MSE - mean square error.

[1] Only 1 value was available for sewage sludge treatment.

[2] Mn data was ln Y transformed. Values are geometric means.

various amended oil sands plots appeared to be more pronounced than those measured for grasses from the subalpine spoil plots (see Table 38).

ii) as for slender wheatgrass, sainfoin plants also had higher levels of P and K in the amended than in the control plots (Table 42), with sewage sludge providing the most P. Amounts of Mn in plants grown in the control, fertilizer and sewage sludge treated plots were significantly greater than in plants from the peat treatment. Compared with the slender wheatgrass (Table 41) sainfoin appeared to have a greater demand for Ca and Mg.

iii) jack pine, like white spruce, demonstrated a much reduced growth rate in comparison with legumes and grasses. As a result, amendation effects on foliage chemical characteristics are difficult to interpret when analyzed after one growing season. However, jack pine did display some root growth during the first growing season, particularly in the sewage sludge amended plots (Table 34), whereas white spruce demonstrated very little root growth over a similar time period (Table 28). Therefore, there was an amendment effect on P levels in jack pine with fertilizer and sewage sludge treatments effecting higher levels of needle P than the control and peat treatments (Table 43). Trees in the peat amended spoil concentrated more Ca than those in the control.

iv) total N levels measured in the foliage of jack pine, slender wheatgrass and sainfoin after two growing seasons are given in Table 44. Shoots of jack pine and slender wheatgrass in the control treatments were too small to be analysed. The results were similar to those obtained for the plant species grown in the subalpine mine spoil (Table 40). Jack pine and slender wheatgrass grown in the sewage sludge and peat treated sand had significantly higher levels of foliage N than those sampled from the control and fertilizer treatments. Sainfoin did not appear to be dependent on N introduced with the amendments since it provides its own N through N_2 fixation.

Table 44. Total N (%) measured in the foliage of selected plant species after 2 growing seasons in amended oil sand.

Treatment	% N Jack pine	 Slender wheatgrass	 Sainfoin
Control	ND	ND	2.3^a
Fertilizer	1.1^a	0.8^a	1.9^a
Sewage Sludge	1.8^b	2.0^b	3.0^a
Peat	1.8^b	2.5^b	2.9^a
df	6	6	6
MSE	0.03	0.14	0.13

Data analyzed by one-way ANOVA. Significant differences located by Scheffé multiple contrasts. Values in each row followed by the same letter do not differ significantly ($p = 0.05$). df = degrees of freedom; MSE = mean square error; ND = not determined.

3.4.3 Correlation of Selected Soil and Foliage Chemical Characteristics

Since the nutrient levels concentrated in the foliage of the test plant species is dependent on the chemical quality of the soil in which the plants were grown, it was decided to test for a relationship between selected spoil and plant chemical parameters. Simple correlation coefficients were calculated between soil and plant P, Fe, Cu and Zn levels measured during the first growing season and the results obtained are presented in Table 45. Like the soil P, N and NO_3-N vs. shoot weight correlation coefficients (see Table 37), the best relationships occurred between soil P levels and P in the foliage of slender wheatgrass, sainfoin and jack pine. There was also a strong relationship between soil Cu and oil sands slender wheatgrass Cu levels. High levels of Cu in the sewage amended sand (see Table 9) may explain this result. The same relationship did not occur for the slender wheatgrass grown in the subalpine sewage treated spoil, presumably because the subalpine spoil type (more clays) in contrast to the nonadsorptive sand tailings may have rendered Cu and some of the other metals unavailable to the plants. There were no strong relationships between the other heavy metals used in the analysis and foliage metal levels indicating that over the short term (i.e. one growing season) there was no significant build-up of metals in the foliage of plants grown in the sewage sludge treated minesoils. The slow uptake of nutrients as a result of the slow growth rate of conifers such as white spruce explains the total lack of agreement between soil and needle nutrient levels. No doubt better relationships between spoil and foliage chemical characteristics would have resulted if:

i) spoil samples for analysis had been removed from the vicinity in which the plants were sampled;

ii) more replicates had been tested;

iii) more replicates within each amendment type had been analyzed.

Table 45. Simple correlation coefficients between soil and foliage chemical characteristics (µg g^{-1}).

		Correlation Coefficients			
Spoil Type	Plant spp.	P	Fe	Cu	Zn
Subalpine	Slender Wheatgrass	.656	.525	-.070	.149
	White Spruce	-.108	-.769	.262	-.083
Oil Sands	Slender Wheatgrass	.528	-.331	.761	.413
	Sainfoin	.672	-.294	-.012	-.024
	Jack Pine	.864	-.129	.162	.007

4. DISCUSSION

4.1 PRAIRIE GRASSLAND MINESPOIL

Mining disturbance of the grassland site (Bow City) resulted in a spoil which had high levels of exchangeable Na, a high SAR, increased clay levels and a decrease in biologically available carbon (the latter being a result of the stripping of the topsoil in the mining process). Power et al. (1978) emphasized that the high exchangeable Na levels, often found in spoils from the Great Plains region, can exert a major effect on infiltration rate and water storage by dispersing the clay particles and restricting entry. This results in increased run-off and less available water for plant growth. Total N and P were not substantially different in the disturbed and undisturbed soils, but it should be emphasized that these results do not reflect the availability of N and P for plant growth. Power et al. (1978) have shown that almost all mine spoils in the Northern Great Plains are deficient in plant available P and biologically active organic N. Plant survival and growth are poor in this spoil type (see Fig. 2, control plots) mainly as a result of adverse seedbed conditions, poor root penetration (due to the impermeable nature of the minespoil), low nutrient status and poor moisture conditions (this would be particularly true at the field site where the annual precipitation is 335 mm).

The addition of gypsum (calcium sulfate) to the grassland minespoil resulted in a small decrease in the SAR coupled with an increase in EC and extractable Na as this ion was replaced by Ca. Gypsum has been widely used for sodic soil reclamation (Power et al., 1978a; Kollman, 1978; Van Rooyen and Weber, 1977; Khosla, Gupta and Abrol, 1979; Safaya and Wali, 1979). According to Power et al. (1978a) treatment of sodic soils with gypsum can reduce the exchangeable Na content 30 to 50 percent in the upper 30 cm of spoil within a few years after treatment. Because gypsum has a relatively low solubility, the length of time required to replace the exchangeable Na with Ca would be dependent on the amount of rainfall received by a site. In the semi-arid climate of the southeast portion of this province, reclamation of a sodic spoil with gypsum would probably be long-term. Prather et al. (1978) have suggested combining gypsum with $CaCl_2$ or H_2SO_4 to reduce the time and leaching required to achieve reclamation. In this study the SAR was not measured after the first sample time as data on the long term effects of the gypsum on the SAR and exchangeable Na levels are not available.

A layer of 15 cm topsoil, spread on the surface of the sodic minespoil, resulted in a much reduced SAR compared with that measured in the spoil (Table 6.6). However, research conducted by Merrill et al. (1980) demonstrated that over an 8 year time span chemical diffusion resulted in significant Na migration into the topsoil spread over minespoil (SAR = 19). They concluded that this Na accumulation could potentially degrade topsoil spread over minespoil with an SAR value of 20 or more and where only 30.5 cm (12 inches) or less topsoil was available for reclamation. In this study, the SAR of the grassland spoil was measured immediately after topsoil addition. In a spoil such as this, where sodicity and salinity appear to be the major factors impeding plant growth, EC and SAR should be monitored frequently. Although sewage sludge application had no immediate effects on the SAR, it is possible that over the long term the high levels of Ca in the sludge would replace some of the Na on the exchange sites; thereby relieving some of the sodicity problems.

Primary production (both root and shoot production) by crested wheat, fall rye, russian wild rye and rambler alfalfa was most stimulated by either sewage sludge or topsoil addition. Other studies have also demonstrated that treatment of a sodic minespoil with topsoil improves plant establishment and productivity (Kollman, 1978; Power et al., 1981; Schroeder et al., 1980; Aldon, 1978) by increasing the infiltration rate, reducing run-off, improving germination (because of better seedbed conditions) and increasing plant survival as a result of better root penetration, improved soil fertility and more efficient water usage by the vegetation. Although 15 cm of topsoil was applied in the present study, recent research by Power et al. (1981) indicates that the reclamation of highly sodic materials (SAR greater than 20), requires a minimum thickness of 90 cm of soil (20 cm topsoil plus 70 cm subsoil) to obtain maximum production of most crops in semi-arid regions. They also emphasized that if the soil material is highly erodible and upward salt migration is a problem, more than 90 cm of soil material may be required. Therefore, if maximum crop production for grazing is one of the aims in reclaiming a sodic minesite in this province, more research would be required regarding the optimum levels of topsoil to be applied taking into consideration the climatic conditions of the mined area. Needless to say, it is necessary that topsoil be stockpiled for future reclamation particularly in remote areas where other amendments are not available and the cost of transporting them to the site would be impractical.

Although sewage sludge has been used extensively for treating agricultural land, it has found only limited use for reclaiming land disturbed by mining. Sopper (1970), Stout et al. (1978), and Berry and Marx (1977) have reported results regarding the use of sewage sludge for reclaiming strip mine spoils in the eastern United States whilst Bradshaw et al. (1978) have used sewage sludge to establish plants on limestone quarry faces in England. Reasons why a nutrient-rich amendment such as sewage sludge has not been more popular for reclamation purposes may be:

i) the remoteness of many minesites resulting in high transport costs of the sludge. Agricultural land is generally within a reasonable distance of sewage sludge lagoons, i.e. near large cities where the sludge is generated.

ii) fear of heavy metal uptake by the vegetation on the minesite which could then lead to the accumulation of metals in the animals grazed on reclaimed minesites.

iii) fear of pathogens which might be present in the sludge.

iv) refusal by mine workers to handle sewage sludge due to its offensive odor, pathogen potential etc.

Nevertheless, in the present study sewage sludge applied at 46 mT ha^{-1} was very effective in promoting the growth of crested wheat, fall rye and russian wild rye. In contrast to the grasses, rambler alfalfa responded poorly to sewage sludge addition. This may have been a result of poor germination and establishment rather than inhibited growth due to the sludge. Also, the amount of sludge applied in this study did not improve seedbed conditions to the same extent as topsoil.

Caution must be exercised, however, when applying sewage sludge since some researchers (Wong *et al*., 1981; Sabey and Hart, 1975; Wollen *et al*., 1978; McCormick and Borden, 1973) have reported inhibition of seed germination and retarded root elongation with the application of liquid sludge or sludge extracts. Sabey and Hart (1975) observed that there was a severe inhibition of germination of sorghum sudangrass and millet when seeded shortly after liquid sludge (a mixture of 50% anaerobically digested primary and 50% aerobically digested waste activated sludges) was incorporated into the soil. However, wheat demonstrated no germination inhibition if seeded 3 mo. after sludge incorporation. They postulated that nonvolatile inorganic salts were not the causative agent of the inhibition since the inhibitory factors were destroyed by combustion at 525°C.

Wollen *et al*. (1978) believed that the intense biological activity which occurred when sludge was freshly incorporated into soil led to reduced oxygen tensions and the release of germination inhibitors such as ammonia and ethylene. McCormick and Borden (1973) in a study designed to measure the chemical changes of the percolate from strip minespoils treated with sewage effluent and sludge observed that many of the plants (hybrid poplar and grasses) they attempted to grow in the sludge-treated spoil died soon after planting or germination. They attributed this result to the presence of toxic concentrations of ammonia, and suggested that after applying large quantities of sewage sludge to a soil, one should wait at least 3 months before planting. As a result, if anaerobically digested sewage sludge is to be used extensively for reclaiming mine sites in Alberta, more data should be collected on the effects of different sludge applications on seed germination, plant establishment and growth. Information regarding seed germination after different sludge application times would also be necessary in order to obtain maximum plant response from the sludge amendment.

The better growth response of the grasses and alfalfa in the topsoil and sewage-sludge treated soil in comparison with that in the gypsum-amended and control plots was also partly a result of the improved N conditions after topsoil addition and the increased quantities of N and P in the spoil after sludge amendation. The sewage sludge appeared to behave as a slow release fertilizer, releasing P and N as it decomposed. More research is required concerning the decomposition and nutrient release rates from the sludge under various soil and climatic conditions in order to predict the nutrient availability to crops over time.

As expected, sewage sludge also increased the levels of Cu, Zn and Pb in the surface soil, but levels were low in comparison with those measured in the sludge prior to application. Most metals added to soil tend to remain in the surface soil with very little movement deeper into the profile (see Williams _et al_., 1980 for a review of the literature regarding trace element movement in the soil). According to Williams _et al_. (1980), most of the metals naturally present in the soil are in forms which are relatively immobile and unavailable to plants. They concluded that after applying sewage sludge annually for 3 years to a loam soil (pH 5.2 to 5.6) at rates of 0 to 225 mT $ha^{-1}yr^{-1}$, the added metals like those originally present in the soil, tended to become immobilized. Only Zn and Cd were not readily chelated and were therefore likely to get into the food chain. Consequently, if only one application of sludge at a reasonably low rate (e.g. 46 mT ha^{-1} as was used in the present study) was to be used to initiate reclamation of a minesite, accumulation of heavy metals by the vegetation would probably not be a problem.

Although the addition of gypsum to the minespoil improved the levels of P available for plant growth, plant response was generally poor. Seedbed conditions (pavement-like and impenetrable) were not significantly different from those in the control plots, so plant establishment appeared to be poor. As stated earlier, the amelioration of a sodic spoil is a slow process dependent on precipitation input and subsequent leaching rates. So, unless gypsum is applied in combination with another amendment such as sewage sludge, one would expect revegetation to be retarded. Revegetation using gypsum could possibly be accelerated if the treated spoil was tilled and seeded every year after amelioration. In the present study, plots were seeded only at the initiation of the project.

With the exception of fall rye, shoot production of the grasses and alfalfa increased in all the treatments over the time span of the study. According to the Manual of Plant Species Suitability for Reclamation in Alberta (Watson et al., 1980), both crested wheat and russian wild rye are long-lived perennials, adapted to dry rangeland or pasture conditions because of their drought tolerance. They have high natural reseeding abilities, are suitable for alkaline soils and have moderate (crested wheat) to high (russian wild rye) tolerance to saline conditions. Crested wheat has excellent establishment qualities and is one of the best bunchgrasses for controlling erosion. In contrast to crested wheat, russian wild rye is often difficult to establish due to poor seedling vigor. In the present study, however, both crested wheat and russian wild rye did not become well established until the second growing season. The slow establishment of crested wheat may have been a result of the dry conditions after the seedlings germinated.

Rambler alfalfa is also a perennial and because of its N_2 fixing capability and drought tolerance is desirable for revegetation purposes. In this study, alfalfa established itself successfully in the topsoil treatment but not in the other treatments. The Manual of Plant Species Suitability for Reclamation in Alberta notes that alfalfa demonstrates poor tolerance to salt at the germination stage - a factor which may explain the poor shoot production results obtained for this plant species in the gypsum and sewage-treated spoil. As mentioned previously the presence of inhibitory factors in anaerobically digested sewage sludge may also have contributed to poor germination of alfalfa seed.

Of all the plant species tested on the ameliorated grassland spoil, fall rye established itself most rapidly and produced a good vegetative cover during the second growing season. Thereafter, it demonstrated very little growth and did not successfully reseed itself. Information on fall rye (perennial ryegrass) presented in the Manual of Plant Species Suitability for Reclamation in Alberta, supports the results obtained in this study - i.e. perennial ryegerass is a rapidly developing, short-lived perennial which will not last more than 3 years on the prairies. It should therefore be considered as a short-term vegetative cover planted mainly to stabilize the soil and thereby prevent surface erosion. According to the Watson _et al_. (1980) manual, perennial ryegrass is not particularly drought tolerant and may therefore have difficulty establishing itself in the semi-arid conditions typical of the shortgrass prairie at the minesite near Bow City, Alta.

From the plant production data collected in this study it appears that fall rye is the best choice if a rapid vegetative cover is required. However, if a long term vegetative cover is the ultimate reclamation goal crested wheat should probably be included in the seed mixture at the time of seeding. A legume, such as alfalfa should also be considered when revegetating a minesite, since it provides a readily available source of N. Jeffries _et al_. (1981) demonstrated that white clover growing in association with grasses on colliery spoil in England was an effective N source with N transfer from the clover to the plant becoming apparent within 22 months. after sowing. Poor establishment of alfalfa on all but the topsoil amendment should be considered when choosing legume species for reclamation of shortgrass prairie minespoil which has been rendered sodic by the mining process.

It should be mentioned that the plant species used in the present study were all introduced forage species which according to Power _et al_. (1978b) are generally more productive than native species but often require a higher level of management including proper seedbed preparation and seeding, good weed control, adequate fertilization and control of grazing time and intensity. Native species in comparison have less intensive management requirements and are more resilient and adaptable under stress conditions such as prolonged drought, intense cold, short growing seasons and to limited nutrient supplies. Therefore, if proper management of forage species is not feasible on reclaimed minesites, native plants should be considered as alternatives for revegetation.

However, Curry (1980), who believes that successful reclamation of arid western lands (those where evaporation exceeds precipitation) should be defined as "restoration of the land to a state in which vegetative cover and nutrient cycles are as "closed" as they were before the man-made disturbance", fears that establishment of native plant communities may not be possible over the short-term without massive inputs of energy over a long time period. Native soils and plant communities may be the result of evolution through a sequence of climatic episodes that may not be reproducible (Curry, 1980). Nevertheless, Power et al. (1978b) have found that, although some native species require three or more years to establish good stands, some of them (some wheatgrass, needlegrass and grama species) are adapted for use on reclaimed land. The establishment of these species on mined land may be the first step in the succession toward a more stable native plant community.

4.2 SUBALPINE MINESPOIL

The major consequence of mining for coal in a subalpine area such as that found at Luscar, Alta. is the removal of the organic mat with its associated fertility, water-holding capacity and desirable seedbed conditions. In the course of excavating a pit for coal, the soil above the bedrock is removed and stockpiled for subsequent revegetation, but according to Macyk (1972) the soil to be stockpiled is sometimes so thin that the incorporation of large quantities of coarse bedrock is unavoidable. The incorporation of excessive quantities of bedrock in the "topsoil" results in revegetation problems (Macyk, 1972).

In the present study, the characteristics of the subalpine minespoil after application of "topsoil" or "regolith" (see Tables 5 and 13) but prior to planting included:

i) a neutral pH (7.0-7.3) due to the calcareous nature of the bedrock.

ii) low N (0.09%) and extractable P (3.4 $\mu g\ g^{-1}$) levels.

iii) elevated total Fe and Mg levels (25,000 and 4,500 $\mu g\ g^{-1}$ respectively).

Organic matter and carbon levels of the minespoil were difficult to measure as a result of coal particle interference. However, visual observation of the spoil material indicated a significant lack of biologically available organic matter. This minespoil was characterized by large (>2 mm) bedrock fragments, low levels of organic matter and plant available nutrients, and neutral pH. Therefore, the subalpine spoil was amended with peat or sewage sludge in an effort to improve the organic matter status, fertility, and water and nutrient retention capacity of the spoil. An inorganic fertilizer treatment was also included in the study since fertilization is commonly used to revegetate subalpine and alpine mine spoils (Macyk, 1972, 1974; Brown et al., 1978; Cherene, 1978).

Some of the chemical and physical characteristics of the sewage sludge included an alkaline pH (8.6), 30% C, 3.9% N and moderately high levels of P, Ca and the heavy metals. The peat used to ameliorate the subalpine spoil was also high in C (36%), and interestingly, contained high levels of N (2.5%) and large quantities of available NO_3. However, P levels were so low in the peat (extractable P = 2 $\mu g\ g^{-1}$ d wt soil) that they were potentially limiting to plant growth.

Immediately after applying fertilizer to the spoil, the pH of the 0-5 cm layer of soil decreased significantly as compared with the control, while the electrical conductivity and extractable cations such as Ca, Mg and K increased. According to Brady (1974), ammonium ions present in the fertilizer will tend to increase acidity when they are nitrified. Also if P is present as $Ca(H_2PO_4)_2$ in the fertilizer granules, it will attract water from the soil resulting in a H_3PO_4-laden solution with a pH of 1.4 moving outward from the granule (Brady, 1974). This reaction may also have contributed to a short-term drop in pH in the fertilized spoil.

If the P fertilizer contained substantial quantities of Ca, it would account for the significant increase in soil Ca levels after fertilization. Sewage sludge and peat application did not alter the pH of the minespoil, but did significantly increase the levels of extractable Ca, Mg and K in the 0-5 cm layer of the soil. The deeper soil layer (5-15 cm) was not affected by amendation, since rototilling of the amendments into the subsurface soil was severely hampered by the presence of large quantities of rock.

Primary production by slender wheatgrass (like that of crested wheat and russian wild rye in the grassland spoil) was most stimulated by the addition of sewage sludge - particularly after the second growing season. Fertilizer application was also highly effective in promoting shoot production by slender wheatgrass. The growth pattern of slender wheatgrass in all but the peat treated spoil was very similar to that demonstrated by fall rye in the grassland spoil - i.e. a peak in primary production after the second growing season followed by a substantial decrease in growth during the third growing season.

According to Watson _et_ _al_. (1980), slender wheatgrass is a short-lived (3-5 years) perennial grass which becomes secondary when planted with long-lived species. The decreased growth by slender wheatgrass in the third growing season may also have been the result of nutrient immobilization in the litter and roots produced during the first two growing seasons. Due to the slow decomposition rate of this litter (46 and 76% wt. remaining after 1 year for stems and leaves respectively) plant essential nutrients such as N and P may have been in low supply during the third growing season.

The significant growth response of slender wheatgrass to the sewage sludge and fertilizer treatments appears to have been a result of the high levels of plant nutrients present in these two amendments. The data presented in Tables 13 and 15 demonstrate that

NO_3-N and extractable P in the subalpine minespoil were significantly increased by the addition of sewage sludge and fertilizer. Substantial quantities of P were still present in the minespoil after the second growing season, particularly in the sewage-sludge treated plots. Although the levels of NO_3-N in the sewage sludge treated soil did not alter significantly from the beginning of the first until the conclusion of the second growing season, the NO_3-N in the fertilized plots had decreased to a level where it was not significantly different from that recorded for the untreated (control) spoil.

As stated previously, sewage sludge may behave as a slow release fertilizer, due to the slow decomposition of the sludge aggregates within the soil matrix. Sewage sludge aggregates sieved from the subalpine spoil two growing seasons after application contained 2.3% TKN, 94 $\mu g\ g^{-1}$ NH_4-N and 84 $\mu g\ g^{-1}$ NO_3-N. The original levels in the sludge would have been approximately 4% TKN, 2% NH_4-N and 0.01% NO_3; hence, substantial quantities of N were still present although the sludge had been decomposing for two years.

Conversely, the N in the fertilizer used in this study was present as ammonium nitrate - a form which makes N readily available to plants and soil organisms. If the N is not immobilized by microbes or plant roots, leaching losses of N, especially when in the NO_3-N form can be severe. In this study levels of extractable P and NO_3-N in unplanted, subalpine minespoil were measured 4 months after fertilization. Levels of P were not significantly altered; however, NO_3-N had decreased sigificantly (presumably as a result of leaching) in the 0-5 cm soil and had not accumulated in the deeper (5-15 cm) soil. Therefore, after the first growing season, the N available to the slender wheatgrass may have become limiting resulting in a decrease in primary production. Macyk (1974) observed that N (100 lbs/acre) applied to a subalpine coal spoil at Grande Cache, Alberta was completely depleted after two growing seasons, and recommended additional N application at the beginning of the second growing season. From the data presented in Table 15, it appears that amendation of a subalpine spoil with sewage sludge would not require additional N at the beginning of the second growing season; whereas, treatment with mineral fertilizer would require additional N applications in the following growing seasons.

The high levels of nutrients available to the slender wheatgrass in the sewage sludge treated plots was reflected in the elemental concentrations measured in the foliage after one growing season (Table 38). A significantly greater amount of P was present in foliage from the sludge amended plots than from the other treatments.

Interestingly, heavy metal levels (e.g. Cu and Zn) measured in plants from the sewage treated spoil treatment were not significantly greater than those measured in plants from the other treatments, although DTPA extractable Cu and Zn levels immediately after amendation were higher in the sludge treated minespoil than in most of the other treatments. As mentioned previously, Williams *et al*. (1980) concluded that metals added to soil through sewage sludge treatment were readily immobilized and made unavailable to plants. It should be recognized, however, that the availability of metals and their uptake by plants is dependent on such factors as chemical composition of the sludge, rate and frequency of application, soil characteristics, and plant species (Zwarich and Mills, 1979). Also, Silviera and Sommers (1977) suggested that the availability to plants of metals in sewage sludge treated soils may be elevated with time through the dissolution of metal precipitates, oxidation of metal sulfides and release of metals complexed with sludge organic matter. However, if only one application of sewage sludge at a fairly low rate is used to initiate reclamation (as was done in this study) it is doubtful that heavy metal accumulation in plants or leaching from the soil system would be a problem. More chemical analyses of the plant species grown in the sludge treated subalpine spoil would be required to analyze the effects of one application of sludge (i.e. 46 mT ha^{-1}) on the long term accumulation of metals in plant foliage.

Although application of a feather moss peat to the subalpine spoil significantly increased N levels (particularly NO_3-N), grass production was not stimulated to the same degree as in the sewage sludge and fertilizer treatments. The extremely low levels of extractable P in the peat (1.5 $\mu g\ g^{-1}$ after application) was probably one factor accounting for the low primary production in this treatment. Peat application also significantly increased levels of Fe, Zn and Ca. Both Fe and Ca can react with P to form insoluble compounds, rendering P unavailable to plants. The initial pH of the peat-amended subalpine spoil, ranged from 6.7-7.2 and according to Brady (1974) the formation of insoluble calcium salts and even more insoluble apatites occurs in this pH range. These chemical reactions may explain the low extractable P levels measured in the peat.

The high levels of NO_3-N (449 $\mu g\ g^{-1}$ - Table 15) and Ca (51,900 $\mu g\ g^{-1}$ - Table 6a) account for the significantly higher concentrations (in comparison with control plots) of N and Ca immobilized in the foliage of slender wheatgrass after one and two growing seasons (see Tables 38 and 40). Although extractable P levels in the peat were exceedingly low, the P concentration in the slender wheatgrass foliage after one growing season was not significantly different from that measured for grass grown in the fertilized spoil. The role of the mycorrhizal fungi in improving the P nutrition of grass growing in a P-deficient peat such as that used in this study would probably be crucial for the survival and health of the plants (Zak et al., 1984).

Alsike clover shoot production, in comparison with that of slender wheatgrass, did not peak during the second growing season, but increased over the 3 year sampling period, particularly in the sewage sludge and fertilizer treated plots. Alsike clover is a short-lived perennial (Watson et al., 1980), but in this study was observed to produce large quantities of seed after the second growing season in all treatments. The seed germinated readily, and this accounted for the increased primary production in all treatments over the 3 years that it was measured. Sewage sludge and fertilizer promoted the greatest shoot production because these amendments contained high concentrations of plant essential nutrients. Although much of the N included in the fertilizer may have leached out of the rooting zone by the end of the second growing season, this would not have affected the growth of alsike clover due to its N_2 fixing capabilities. Measurements of N fixation (using the acetylene reduction technique) demonstrated that during the second growing season, alsike clover roots in all treatments were nodulated and had the capacity to fix N_2 (Parkinson et al., 1980). The %N measured in the foliage of alsike clover after the second growing season demonstrated no significant effect of treatment on plant N levels (see Table 40), providing further evidence that even under the low N conditions found in the untreated subalpine spoil, a legume such as alsike clover is capable of fixing enough N_2 to meet its N requirements.

Application of peat to the subalpine spoil did not stimulate shoot production of alsike clover in comparison with shoot production in the control treatment. Although no data were collected regarding establishment success of alsike clover in the peat treated spoil, field observations indicated that during the first growing season clover seedlings did not establish themselves readily, and once established demonstrated very poor growth. It was postulated that the low extractable P level (1.5 $\mu g\ g^{-1}$) in the peat was partially responsible for the poor growth response by the clover. A greenhouse pot experiment to study the effects of added P on the growth of alsike clover in the peat treated subalpine spoil, demonstrated that additional phosphorus (272 μg P g^{-1} peat) significantly increased plant production by alsike clover and also enhanced flower production (S. Visser, unpubl. data). No flowers were produced by plants grown in the untreated peat, indicating the significance of adequate P availability to flowering and seed formation. The lack of seed production as a result of low P levels would influence the reseeding success of alsike clover (Parkinson et al., 1980). Watson et al. (1980) also note that alsike clover requires moderate amounts of P for satisfactory growth. As mentioned previously, a legume should be grown in association with grasses when reclaiming minespoils to provide an effective and long term N source. Under the conditions imposed in this study, alsike clover established and reseeded itself successfully when adequate levels of P were available. If it has the ability to compete with grasses, it is recommended for use on reclamation sites.

In contrast to the slender wheatgrass and alsike clover, white spruce demonstrated no significant shoot growth until the fourth growing season (see Fig. 3). Because of its slow growth rate, no effects of amendation on shoot weights were recorded until the end of the fourth year when spruce in the sewage sludge and peat treated spoil were heavier than those in the fertilized and untreated spoil. The probability that sewage sludge provided adequate levels of available nutrients to the spruce seedlings over the long term (in this instance, 4 years) would explain the better growth of spruce in the sludge-treated spoil in comparison with that measured in the control spoil. Although fertilizer treatment introduced adequate levels of available N and P to the minespoil when the trees were planted, the slow growth and uptake of nutrients by white spruce resulted in the loss of nutrients (particularly N) through leaching before the trees could immobilize them. Therefore, by the third growing season, nutrient levels in the fertilized spruce subplots were probably similar to those in the control subplots resulting in a growth response similar to that measured in the untreated minespoil. Therefore, if fertilizer is to be used to stimulate growth of slow-growing species such as white spruce, its application should be "tailored" to meet the demand of the tree i.e. continuous application over the long term.

In contrast to the results on shoot production obtained for slender wheatgrass and alsike clover grown in the peat amended spoil, shoot production by white spruce planted in the peat treatment was not significantly different from that measured in the sewage treatment at the conclusion of the fourth growing season. The different response of the grass and clover to the peat treatment compared with that observed for the spruce may be explained by:

i) the nutritional demand by the white spruce in its early stages of growth is much less than that for a fast growing grass or legume. The slender wheatgrass and clover may have taken up many of the nutrients available in the peat and immobilized them in dead plant litter and roots. On the other hand, the slow growing white spruce are incapable of rapidly immobilizing nutrients; therefore these nutrients, with the aid of mycorrhizal fungi, are available over a much longer term.

ii) it is possible that over the term of the study, some decomposition of the peat may have occurred, thereby releasing nutrients such as P which stimulated tree growth after the third growing season. Microbial respiration and microbial biomass C measurements for the peat treated subalpine spoil demonstrated a significant increase between the beginning of the first growing season and end of the second growing season, indicating that some decomposition of the peat may have occurred after its application to the spoil. More research would be required to determine whether freshly excavated peat decomposes after spreading on a spoil surface and, if so, at what rate and to what degree.

A chemical analysis of the white spruce needles after one growing season showed no real amendment effects on nutrients taken up by the tree. The slow growth and low nutrient uptake by the tree during the first growing season would explain the lack of treatment effects on needle chemical composition. Foliage analysis over the long term would be required to assess the effects of the various treatments on nutrient levels in white spruce.

The percent survival of white spruce over the first two growing seasons was high (84-90%) and was not significantly influenced by treatment.

According to Selner (1977), white spruce is considered to be the best conifer species for reclamation of coal mined areas in the eastern slopes. However, if white spruce or other woody species are planted in areas which were originally seeded with grasses, tree growth may be reduced. Research by Fales and Wakefield (1981) demonstrated that turf grasses such as perennial ryegrass, red fescue and Kentucky bluegrass significantly reduced the growth of woody species such as flowering dogwood. They attributed the reduced growth to direct competition between the plant species for available N, and also to the production of allelopathic substances by the turf grasses. Also, if primary production of grasses has been encouraged by regular fertilizer applications, a thick mat of dead litter may develop since decomposition in subalpine areas is retarded by low temperatures. This litter layer offers protection for small rodents which multiply and can become a menace to young tree seedlings.

In comparison with the slow growing white spruce, willow demonstrated very rapid shoot growth particularly in the sewage sludge amended spoil. Response by the willow to the high levels of nutrients added to the minespoil in the form of sewage sludge and fertilizer was evident after the first growing season when shoot production in these two treatments was significantly greater than that in the control and peat treated plots. Shoot production continued to be highest in the sewage sludge amended spoil over three growing seasons, whereas shoot production in the fertilized plots was significantly less than that measured in the sludge treatment after the first growing season. As mentioned previously, loss of N through leaching from the fertilizer treated spoil during the first growing season would be one possible explanation for decreased growth during subsequent growing seasons. Additional fertilizer applications would be required to maintain the rapid growth of a shrub such as willow in the subalpine coal spoil. However, shoot production in the sewage sludge treatment was maintained at a high level over the three growing seasons - presumably as a result of nutrients being released over a long term from the slowly decomposing sludge aggregates. The lack of easily available P in the peat amended spoil may account for the low growth response by willow to this treatment.

Although percent survival of willow was high (91-97%) over the first 2 years and was not significantly affected by treatment, percent dieback after the second winter was significantly greater in the peat amended spoil than in the other treatments. This dieback may have been a result of very dry fall weather which dried the peat creating drought conditions over the winter. *Salix* spp. such as *Salix glauca* L. are not considered to be very drought tolerant (Watson *et al*., 1980). However, the dieback did not appear to affect subsequent growth and survival of the plants.

Willow spp. are known as important sources of browse for moose, bighorn sheep, elk and mule deer (Watson *et al*., 1980). In this study evidence of browsing by deer was apparent after the second growing season, particularly in treatments where branch growth during the previous growing season had been high. The level of browsing encountered in this study did not appear to affect subsequent shoot production, although quantitative data would be required to substantiate this observation. Because shrub species such as willow are prone to browsing and dieback, height measurements to assess effects of various treatments on shoot growth can be very misleading. Shoot weight measurement after destructive sampling is a more accurate method for obtaining data on plant response to amendation.

4.3 OIL SANDS MINESPOIL

Surface mining of the Athabasca Oil Sands deposit involves the stripping of the vegetation and layers of clay, sand, gravel and boulders to expose the bitumen-bearing sand. If large quantities of peat and clay overlie the oil sand deposit, these are removed and stockpiled for future reclamation of the oil sand tailings. The oil extraction process involves the use of hot water, steam and caustic soda to remove the bitumen from the sand. This process results in the production of large volumes of an alkaline, aqueous suspension of clays which are stored in large tailings ponds. The ponds are dyked with the extracted sand. As a result of the harsh extraction process, the characteristics of the sand tailings include the following:

i) sand content is high (96%) while silt (2.7%) and clay (1.0%) contents are low resulting in low water holding capacity (Turchenek, 1976). In the present study the sand was found to be fine-grained with 86% of the sand grains falling in the 125-500 μm size range. The sand also appears to be hydrophobic, therefore not rewetting evenly (McCoy et al., 1976; Turchenek, 1976; Takyi et al., 1977) - a factor which leads to erosion problems particularly during heavy rainfall (Takyi et al., 1977).

ii) pH of fresh tailing sand is alkaline but tends to decrease to neutrality after one or two years of exposure (Lesko, 1974). Turchenek (1976) recorded a pH of 9.2 for tailing sand whilst Takyi et al. (1977) and McCoy et al. (1976) recorded pH values of 6.4 and 6.6 respectively. In the present study, the pH of tailing sand from the control plots was recorded as being approximately neutral (7.3-7.5) indicating that this particular sand had been exposed for some time prior to its shipment to Calgary from the Suncor site. Sodium (0.8 me l^{-1}) and E.C. (0.4 ms/cm) measurements of the sand were low - an observation also made by Takyi et al. (1977) and McCoy et al. (1976). Thus plant growth problems as a result of the NaOH used in the oil extraction were not encountered in this present study.

iii) the nutrient status is very low (Massey, 1972; Turchenek, 1976; McCoy et al., 1976; Takyi et al., 1976). Nutrient levels of the extracted oil sand used in this study were low (i.e. NO_3-N, 0.1 $\mu g\ g^{-1}$; extractable P, 1.2 $\mu g\ g^{-1}$). According to Turchenek (1976) the infertility of the tailing sand is due mainly to the lack of organic matter and inorganic colloids. The organic content of the sand in this study was found to be 0.2-0.4% which is similar to that obtained by Turchenek (1976). Due to the low organic matter content, the CEC, nutrient retention and water holding capacity would also be expected to be low, resulting in leaching problems if nutrients such as fertilizer are added.

Since the tailing sand has been shown to be mainly sand with associated low fertility and low water holding capacity, successful reclamation of this minespoil would require the addition of amendments to raise the nutrient status, nutrient retention and water holding capacity. Therefore, in this study, the following amendments were applied to oil sand tailings:

i) inorganic fertilizer to improve the fertility of the sand and thereby promote plant growth.

ii) sewage sludge to improve the fertility of the sand and also to increase moisture and nutrient retention.

iii) feather moss peat which, because of its high organic matter content, would significantly increase the nutrient and moisture retention, and CEC of the sand. It would also act as a source of plant nutrients.

The application of inorganic fertilizer to the sand resulted in a significant drop in pH (6.4) as compared with the control (7.3). This was also observed for the subalpine spoil, and may have been caused by the same factors as discussed for that spoil type. Levels of extractable K, extractable P and NO_3-N in the sand were significantly higher after fertilizer application. However, approximately 4 months after fertilizer application, extractable P and NO_3-N measurements had decreased significantly in the 0-5 cm depth of sand in the unplanted pathways (see Table 19). These nutrients had not accumulated in the 5-15 cm depth of sand, indicating the high leachability of the sand.

Addition of sewage sludge to tailing sand also improved the fertility of the sand by increasing the levels of extractable cations, extractable P and NO_3-N. Amounts of DTPA extractable Cu and Zn in the sand were also significantly greater after application of the sludge; particularly in the surface soil. Because of the organic nature of the sewage sludge, the CEC, moisture and nutrient retention capabilities of the sludge-amended sand were probably also improved particularly in and near the sewage sludge aggregates.

Amendation of the extracted sand with peat resulted in increased levels of DTPA extractable Fe and Zn and also NO_3-N. However, as was the case for the subalpine spoil, amounts of extractable P in the peat-treated sand were not significantly different from those measured in the untreated control. McCoy et al. (1976) mentioned that decomposed peat has a low extractable P content, suggesting that supplemental P would be required to promote and maintain a vegetative cover on this particular amendment.

Amendation effects on shoot production of slender wheatgrass were highly variable in all treatments. This variability may have been the result of the patchy distribution of nutrients throughout the sand particularly in the sewage sludge treated mine tailings. Since the sand is so nutrient-poor, the sewage sludge aggregates (which resulted after the liquid sludge had dried on the sand surface) probably behaved as nutrient-rich islands around which plant growth was stimulated. Also, sewage sludge may have inhibited seed germination and shoot growth during the early stages of plant development for the same reasons outlined in the grassland spoil discussion. To decrease the variability resulting from the uneven nutrient conditions, more samples should have been processed, but this was not feasible in this study due to the limited size of the plots. However, slender wheatgrass did tend to respond more favorably to the sewage sludge treatment (in comparison with the other treatments) particularly during the second growing season when primary production peaked. McCoy et al. (1976) obtained similar results when spent sand treated with sludge and NPK fertilizer; sludge significantly increased barley yield in comparison with the yield produced on spent sand and NPK fertilizer alone. The high levels of nurients added with the sludge (particularly N and P), the relatively long term release of these nutrients and the nutrient retention capacity of the sludge (resulting in an improved CEC for the highly leachable tailing sand) are all factors which would contribute to stimulated plant growth in sludge-treated sand.

The improved fertility of the sewage-sludge amended tailing sand was evident in the foliage analysis of slender wheatgrass after one and two growing seasons i.e. concentrations of N and P were significantly greater in plants from the sludge treated sand than from the control. Levels of heavy metals (Cu and Zn) were not significantly higher in plants from the sewage sludge amended subplots than in plants from the control subplots, again demonstrating that over the short term (1 year) and with a low sludge application rate (46 mT ha^{-1}) heavy metals were not concentrated in this particular grass. The metals are probably strongly complexed with the sludge organic matter and released over the long term as the sludge aggregates degrade.

A comparison of the foliage analysis of slender wheatgrass grown in the sewage sludge treated subalpine and extracted sand minespoils, indicated that more nutrients (particularly P) were available to the grass in the sludge treated sand than in the sludge-treated subalpine spoil. The low CEC (2.9 - Takyi et al., 1976) and associated low nutrient retentitivity of the tailings sand implies that many of the nutrients released from the sludge into the soil solution around the sand particles would be directly available to the plant roots, rather than being immobilized by clays or organic material. Since nutrient availability is greater under these conditions, elemental concentrations in the foliage of plants grown in sludge amended sand would be expected to more closely reflect nutrient availability than would plants grown in soils possessing nutrient complexing or retention properties. The high availability of nutrients and other elements in the soil solution of the tailings sand could also lead to more pronounced toxicity effects if too much sewage sludge were applied, thereby resulting in reduced plant growth.

Fertilizer and peat amendation of the extracted oil sands, also stimulated slender wheatgrass shoot production, but not to the same degree as did sewage sludge. As mentioned previously, many of the nutrients applied through the fertilizer had probably leached out of the rooting zone during the first growing season, except for those nutrients immobilized in the shoots and roots of the grass. Therefore, primary production after the first growing season may have been limited by lack of nutrients. In a growth chamber experiment, Takyi et al. (1976) observed that fertilizer applied to tailing sand improved the growth of legume-grass mixtures. They concluded that plants could be grown on tailing sand if maintained with a good fertilization program. Successful revegetation of tailings sand using fertilizer amendation would require more than one application of fertilizer (as was used in this study). Repeated fertilizer applications require careful management particularly if the reclamation goal entails high yields of forage crops, but also if the aim is to encourage the establishment of native plants which would initiate succession toward a more stable plant community.

Peat also stimulated growth by slender wheatgrass when compared to growth in the unamended tailing sand. However, the low level of extractable P in the peat (after the second growing season, 0.8 μg P g^{-1} peat was measured in soil from the slender wheatgrass subplots) probably limited growth of the grass. As stated earlier, NO_3-N levels were high in the peat and this was reflected in the amount of N measured in slender wheatgrass foliage (2.5%) after the second year. The quantity of P measured in the slender wheatgrass shoots was not significantly different from that measured in plants grown in the unamended sand. As for the slender wheatgrass grown in the peat treated subalpine spoil, this result might be explained by the presence of efficient vesicular-arbuscular mycorrhizal fungi in the grass roots. Takyi _et_ _al_. (1976) observed that fertilizer applied to a tailings sand-peat mixture considerably improved the growth of a legume grass mixture, whilst McCoy _et_ _al_. (1976) found that barley yields were significantly higher in a spent sand plus peat plus NPK mixture than in a spent sand plus NPK mixture. It appears, therefore, that some additional P would be required to improve plant establishment and growth in a NO_3-N rich, but P deficient peat such as that used in this present study.

In agreement with Vaartnou (1974), seed of slender wheatgrass germinated readily in the untreated tailing sand in the plots. However, the low nutrient status of the sand severely limited plant growth. The pattern of shoot production by slender wheatgrass in the sewage sludge and fertilizer treated sand was similar to that observed for slender wheatgrass in the subalpine spoil - i.e. a peak during the second growing season followed by a significant decline in the third growing season. That this species is a short-lived perennial (Watson _et_ _al_., 1981), and that many of the plant essential nutrients had become limiting as a result of immobilization in the slowly decomposing litter may be factors which explain this growth pattern. It should also be mentioned that the seed produced by the slender wheatgrass (particularly in the sludge treated sand) germinated readily in the pathways, but not in the grass vegetated subplots.

Shoot production by sainfoin was, like that of the slender wheatgrass, most stimulated by sewage sludge amendation. Shoot production was consistently higher in the sludge treated sand than in the other amendments over the 3 year term of the study. Again the significantly better fertility of the sand (particularly N and P) over the long term after sludge application would account for the success of this species in this treatment.

Although sewage sludge significantly stimulated growth by sainfoin, it was observed that over the term of the study, $N_2(C_2H_2)$ fixation by this species was less than that measured for plants from the other three treatments. Measurements made during the third growing season demonstrated that reduced $N_2(C_2H_2)$ fixation by sainfoin in the sewage treatment may have been a result of high levels of nitrate or high levels of heavy metals around the sewage sludge clumps where roots tended to concentrate. Munns (1977) in his review of mineral nutrition and the legume symbiosis noted that various studies have demonstrated that high NO_3-N levels inhibit nodule development, retard nodule growth and reduce the rate of N_2 fixation. Vesper and Weidensaul (1978) found that 1-5 ppm Cd, Ni and Cu reduced nodule numbers, N_2 fixation or both. Zinc also reduced nodulation but inhibited N_2 fixation only at the 5-10 mg L^{-1} levels. As mentioned previously Cu levels in the sewage sludge-amended tailing sand (0-5 cm depth) were significantly greater than in the other treatments. The low CEC of the sand may have resulted in a greater availability of metals such as Cu in the soil solution around the roots. The increased Cu level combined with the high N levels in the sludge treated plots may have been factors contributing to inhibited N_2 fixation in this treatment.

Fertilizer (NPK) amendation of the tailing sand also stimulated shoot production by sainfoin, but only during the first growing season. A decrease in the fertility of the sand as a consequence of fertilizer leaching or nutrient immobilization in undecomposed shoots and roots may account for these results. Because sainfoin has the capacity to fix N_2, loss of N from the sand should not seriously affect plant growth. However, excessive loss of P through leaching so that P becomes limiting may affect efficient nodule function. Munns (1977) mentioned that there is evidence in the literature that P fertilization improves nodulation and that nodule function may require more P than the growth of the host plant. Therefore, limiting P levels in the fertilized tailing sand after one growing season may also have indirectly affected primary production. Also excessive quantities of N in the first growing season may have delayed nodulation and associated N_2 fixation until the second growing season, when low P levels may have decreased the N_2 fixing efficiency of the nodules.

In short term pot experiments, Takyi _et_ _al_. (1976) showed that slender wheatgrass-sainfoin mixtures performed considerably better in fertilized (NPK) tailing sand, but that high soil fertility gave a competitive advantage to the grass. Vaartnou (1976) also reported that productivity of various native legumes planted in tailings sand was greatly enhanced when treated with fertilizer. However, germination tended to be delayed if fertilizer was added at the time of seeding. According to Brady (1974) fertilizer salts placed directly under the seeds can result in injury to the plant as a result of the upward movement of NO_3 and K salts by capillary water. Also ammonium fertilizers may release ammonia which has been shown to inhibit seed germination (see grassland spoil discussion). As a result, fertilizer application to the highly leachable tailing sand would probably be more practical after germination of the seed when nutrient uptake by the plants can occur.

Peat treatment of the tailings sand also stimulated primary production by the sainfoin although results were highly variable. Vaartnou (1976) in a greenhouse pot experiment observed improved shoot height of three native legume species when peat was added to tailing sand. He also found that more peat mixed into the tailing sand led to greater shoot height by American milk vetch, showy locoweed and golden bean and that the addition of fertilizer had no effect. In contrast, Takyi et al. (1976) demonstrated that peat addition to tailings sand slightly improved the yield of a slender wheatgrass-legume mixture, but that the addition of NPK fertilizer substantially increased shoot and root production. Since the peat and tailing sand used in this present study contained very low quantities of extractable P, addition of a P fertilizer (N was not required due to the high levels of NO_3-N in the peat) would no doubt have resulted in improved growth and N_2 fixation capability by the sainfoin, particularly during the first growing season. As was the case for the subalpine spoil, microbial respiration and biomass of the peat amended tailing sand also demonstrated an increasing trend with time - a factor which may have resulted in the mineralization of some of the bound P in the peat, thereby stimulating growth during the second and third growing seasons.

Although germination of sainfoin seed was not inhibited in the untreated (control) tailing sand, establishment was very low. However, a few plants did survive the first growing season and were observed to grow remarkably well during the second growing season, despite the high nutrient stress in the tailing sand. Some of these plants were included in the random quadrats which were clipped for primary production estimates. In the process of sampling the roots at the conclusion of the second growing season, it was observed that sainfoin in the control subplots had rooted so deeply that they had grown out of the sand layer and into the gravel underneath the sand. The better nutrient conditions in the gravel accounted for the success of the surviving plants and explains the shoot production results obtained after the first year in the control subplots (see Table 32).

Sainfoin is a short-lived perennial which re-establishes itself mainly by seed. Sainfoin seed was observed to germinate fairly readily, but production of seed may have been severely limited by selective grazing of sainfoin flowers by deer, regardless of treatment.

The %N immobilized in the sainfoin foliage after the second growing season ranged from 1.9% in the fertilizer to 3 % in the peat and was not significantly affected by treatment. The N_2-fixing ability of the sainfoin or the high levels of N available in some of the treatments would account for the lack of treatment effects.

Jack pine, like slender wheatgrass and sainfoin, was most productive in the sewage sludge-treated sand tailings, presumably for the same reasons as those mentioned for the legume and the grass. Berry and Marx (1977) recorded stimulated growth by loblolly pine seedlings (Pinus taeda L.) on sewage sludge treated kaolin spoil, but that high seedling mortality occurred when the sludge application was 138 or 275 mT ha^{-1}. This mortality was attributed to toxic substances in the sludge. As noted previously, there is evidence in the literature which suggests that ammonia released from sewage sludge can severely inhibit root elongation. High ammonia levels in the heavy applications of sludge used by Berry and Marx (1977) may have been one of the toxic substances causing seedling mortality. In the present study seedling survival of jack pine was higher in the sewage sludge amended sand (97%) than in the other treatments; hence the sludge application rate (46 mT ha^{-1}) was low enough that it stimulated growth, but did not affect survival. Since shoot production (and associated response to amendation) by jack pine was minimal during the first growing season, results on chemical analyses of the needles should be treated with caution. The %N in the needles after two growing seasons was significantly greater for trees grown in the sludge treated sand than in the fertilized sand suggesting that high levels of N were available to the trees over the first two growing seasons. Estimation of heavy metal immobilization by jack pine as affected by sewage sludge treatment would require more analysis over the long term.

Fertilizer also stimulated jack pine productivity but the stimulation was considerably less than that recorded for the sewage sludge. The low growth response by jack pine during the first growing season was similar to that recorded for white spruce (subalpine spoil). The slow growth of the trees coupled with the high leachability of the tailing sand, resulted in a considerable loss in nutrients before the trees had developed a large enough root system to take advantage of the improved fertility. The significantly lower level of N in the foliage of jack pine after two growing seasons, compared with that recorded for trees in the sludge and peat treated sand suggests that N in the fertilized plots may have become limiting after the first year. Bengston et al. (1978) found that growth of loblolly pine seedlings on an infertile, readily leached coal mine spoil was greatly increased by NP fertilizer application, but that maintenance of rapid tree growth required inputs of N on a continuing basis. Experiments conducted to study the effects of interseeding loblolly pine with a legume (Lespedeza cuneata) showed that by the sixth year, growth rate and N status of the trees planted with the legume was almost equal to trees which had received two 112 kg ha^{-1} increments of supplemental N. Perhaps sainfoin interseeded with jack pine would produce similar results. Bengston et al. (1978) also demonstrated that grasses (fescue and bermuda grass) interseeded with pine somewhat reduced pine growth and attributed this result to competition for moisture. As stated in the discussion on the subalpine site, evidence provided by Fales et al. (1981) indicated that reduced growth by woody perennials planted amongst turf grasses may involve the production of allelopathic substances by the grasses. The results obtained by these authors should be considered when planting trees and shrubs in areas already revegetated with grasses.

After sewage sludge, peat was the next most effective amendment for promoting shoot growth by jack pine, particularly during the third growing season. Interestingly, white spruce in the peat treated subalpine spoil demonstrated a very similar growth response. The nutrient retentivity properties of the peat and the high level of NO_3-N in the peat coupled with a probable mineralization of bound P from the peat through microbial action may all be factors contributing to the improved growth by jack pine during the third growing season in the peat treated spoil. The large quantity of available N in the peat was reflected in the %N in the needles after two years growth when no difference was found between %N in trees grown in sludge treated sand (1.8%) and trees grown in peat treated sand. Survival by jack pine in the peat amended sand was high (88%).

Although survival of jack pine after the first growing season was high in the untreated sand, growth was negligible due to the low fertility of the tailing sand. Presumably during the first growing season jack pine seedlings in the control subplots survived on nutrient reserves present in the peat plug in which they were grown prior to outplanting. Once these reserves were spent many of the seedlings died as was evident by their 54% survival after the second winter.

After two growing seasons, bearberry in the sewage sludge and peat amended tailing sand had produced significantly heavier shoots than bearberry in the fertilized and untreated sand. Like the conifers used in this study, bearberry is a slow growing plant (in comparison with the grass and legume) and, therefore, does not have the capacity to rapidly immobilize readily available nutrients until it becomes well established and has produced a substantial root mat. As a result, bearberry would not have benefited to the same degree as the grasses from the mineral fertilizer applied prior to planting since its root mat was negligible during the first growing season, allowing nutrients to be readily leached from the soil system. The slow release of nutrients from the sewage sludge and the nutrient retention capacity of the peat resulted in more nutrients being available in these two treatments during the second growing season when the bearberry could respond to them. It is interesting to note that all the woody perennials tested in this study (i.e. white spruce, willow, jack pine and bearberry) demonstrated very poor growth (i.e. shoot production) during the first growing season. No real growth and associated response to nutrients occurred until the second growing season when the plants had become well-established. This suggests that amendments, which provide slow release of nutrients (e.g. sewage sludge) would be more beneficial to these species over the long term than applications of highly available nutrients in the form of inorganic fertilizers. This would be particularly true when amending infertile, coarse grained minespoils where nutrient loss through leaching can be a major problem. In contrast to the other woody species tested in this study, bearberry performed remarkably well in the P deficient peat treatments. Since bearberry is generally found on nutrient poor, coarse-textured soils (Watson et al., 1980), its demand for P may not be as great as it is for other plant species; hence it was not as sensitive to the low P levels in the peat and, as a result, successfully colonized the peat treated tailing sand.

Although bearberry, once established, appears to grow well it was very susceptible to winter kill particularly during the first winter after planting. Also, seedling mortality was found to be treatment related with seedlings planted in tailings sand treated with fertilizer or sewage sludge demonstrating the poorest survival (31% and 38% respectively). It is possible that, if bearbery is a species with low nutrient requirements, the high levels of nutrients available in the sludge treated and fertilizer sand during the first growing season may have been toxic to the seedlings as they established themselves. Once established and growing, they seem to respond favorably to high nutrient levels as demonstrated by their growth response in the sludge treated subplots during the second growing season. More research is required to determine the optimum conditions for the establishment, growth and survival of bearberry.

5. CONCLUSIONS

With the exception of the legumes (which have been introduced from Europe), all of the plant species tested in the subalpine and oil sands minespoil are native to North America. All the species, once they had established themselves, did reasonably well to very well in the variously treated minespoils. Generally, sewage sludge treatment of the minespoils promoted the best growth response from all tested plant species over the term of the study (i.e. 3-4 yrs). The high nutrient content of sewage sludge and the slow release of these nutrients over a relatively long period of time suggests that this amendment (if readily available) be strongly considered for the reclamation and revegetation of infertile, highly leachable minespoils. Even at the low application rate (once at 46 mT ha^{-1}) used in this study, sewage sludge was still stimulating growth of many of the plant species up to five years after its application.

Peat was considered the next best amendment for revegetation purposes, although the low extractable P levels in peat are believed to have inhibited the growth and establishment of some of the test plant species particularly during the first growing season. However, peat did improve the nutrient and water retention properties of the two spoil types and with an initial addition of inorganic P may be the best amendment over the very long term.

Because the minespoils (particularly oil sand tailings) are highly leachable, inorganic fertilizer treatment resulted in only very short term (one growing season) stimulation of plant growth. Over the long term, many applications of fertilizer would be required to ensure continued growth and maintenance of a vegetative cover. It would be much more practical to use fertilizer in conjunction with an organic amendment such as peat if long term revegetation by a particular plant species is the reclamation goal.

Of all the plant species tested, slender wheatgrass appeared to be the shortest lived and the least successful in reseeding itself within the grass subplots under the conditions set in this study. It is therefore considered a short term cover species, perhaps useful for erosion control. Because it is short lived, slender wheatgrass may be a good species to plant prior to the introduction of the longer lived woody perennials such as white spruce or jack pine, particularly if a managed forest is the aim of reclamation. However, in a situation such as this conditions should be such as to allow efficient decomposition of the grass detritus with attendant nutrient recycling. Prior to planting trees in areas originally revegetated with grasses it should also be kept in mind that a grass cover (particularly a healthy grass sward) may inhibit the growth of trees through competition for water and nutrients or through the production of allelopathic substances.

Both legumes (i.e. alsike clover and sainfoin) performed very well under the climatic conditions found in the Calgary area. Through their ability to fix atmospheric N_2, they have the capacity to improve the N status of a minespoil over the long term. Since minespoils are notoriously low in N, legumes such as alsike clover should be considered an essential component of a revegetation program and care should be taken that they become well established. If a reasonably long term shrub cover is required for revegetating and stabilizing a minespoil, willow should be considered. It grows rapidly and has the ability to withstand stresses such as heavy browsing since it can readily resprout. If the aim of a revegetation program is to initiate the early stages of a plant succession towards a more stable plant community, willow should be considered since it is a common invader of naturally disturbed areas. Because willow (along with the grasses and legumes) is fast growing it can readily immobilize nutrients in litter and roots; thereby conserving nutrients for future plant growth rather than losing them from the soil system through leaching. This process is instrumental in the initiation of soil formation - a necessary step if succession is to take place. Bearberry, once established, also grew well, but its high mortality may make it an impractical species for rapid revegetation of a mined area.

6. SUMMARY

6.1 PRAIRIE GRASSLAND MINESPOIL

i) The prairie grassland minespoil was characterized by moderately high levels of clay and SiH and exchangeable Na, a high SAR and low fertility.

ii) A layer of topsoil, 15 cm thick, spread on the surface of this minespoil was most effective in reducing the high SAR and improving seedbed and fertility conditions. Sewage sludge amendation also improved the nutrient status of the spoil, but was not effective in reducing the SAR over the short term.

iii) Fall rye and crested wheat were most productive in the sewage sludge and topsoil treated spoil; russian wild rye performed best in the sewage sludge treatment, and topsoil promoted the most growth by rambler alfalfa. In general, gypsum was the least effective in promoting plant growth in the grassland minespoil.

iv) Plant production by crested wheat, russian wild rye and rambler alfalfa increased steadily over the three year study term, particularly in those treatments which stimulated growth. However, the shoot weight produced by fall rye peaked in the second growing season and then decreased sharply in the third growing season, suggesting that this plant species is very short-lived, while the other three plant species can be considered long-lived perennials.

6.2. SUBALPINE MINESPOIL

i) Removal of the organic mat in the process of mining for coal in the subalpine region results in a minespoil which is dominated by large rock fragments. It is therefore characterized by low levels of organic matter and plant required nutrients and a poor water holding capacity. The pH approaches neutrality.

ii) Sewage sludge amendation of the subalpine spoil was highly effective in promoting shoot production of the test plant species, i.e. slender wheatgrass, alsike clover, white spruce and willow, over the term of the study. Sewage sludge significantly improved the fertility of the spoil, and appeared to be releasing nutrients over the long term. After two growing seasons, substantial quantities of N were still present in the sludge aggregates sieved from the spoil.

iii) Addition of inorganic fertilizer to the spoil stimulated shoot growth of slender wheatgrass and willow, but not to the same degree as did sewage sludge. There was some evidence that leaching losses of N from the fertilized spoil, particularly during the first growing season, may have significantly affected primary production in subsequent growing seasons. Interestingly, alsike clover performed as well in the fertilizer treatment as in the sewage sludge treatment. Since alsike clover has the capacity to fix its own N, it would be less sensitive than non N_2 fixers to leaching losses of N from the fertilized spoil.

iv) Although the peat improved seedbed conditions and introduced large quantities of NO_3-N, it was severely lacking in plant available P. Therefore, the grass, clover, and willow performed poorly in this amendment, in comparison with their growth response in the sludge and fertilizer treatments. Alsike clover was observed to be particularly sensitive to the low P levels. In a greenhouse pot experiment, clover grown in the peat fertilized with superphosphate produced significantly larger plants and more flowers than clover grown in the untreated peat.

v) White spruce performed as well in the peat amended spoil as in the sewage sludge treatment, but not until the fourth growing season. Since white spruce is extremely slow growing, it requires a long term supply of nutrients which is not readily lost through leaching. Sewage sludge appears to behave as a slow release fertilizer, while there is some evidence that over the term of the study unavailable forms of P bound in the peat may have been mineralized through microbial action.

vi) Although sewage sludge application increased levels of DTPA extractable Cu and Zn in the spoil, these metals had not concentrated in the foliage of slender wheatgrass or white spruce after one growing season.

vii) The high levels of NO_3-N present in the peat amendment were reflected in the significantly higher quantities of this nutrient measured in the foliage of slender wheatgrass and willow grown in the peat-treated minespoil. Foliage N of alsike clover was not affected by treatment.

viii) Extractable P measurements in the various treatments demonstrated a strong relationship to shoot production of slender wheatgrass and alsike clover during the first growing season. However, soil extractable P levels were not highly correlated with foliage P of slender wheatgrass during the first growing season.

ix) Primary production by slender wheatgrass peaked during the second growing season, then declined sharply during the third growing season, suggesting that this grass, like fall rye, is a short-lived perennial suitable only as a short term plant cover.

x) The fast growing plant species, i.e. slender wheatgrass, alsike clover and willow can play a major role in rapidly immobilizing nutrients (which are otherwise lost through leaching), in the leaves and root mat. This process is important if succession towards a self-maintaining vegetative cover is to be initiated in the nutrient-poor conditions of a minespoil. White spruce, which is a very slow growing species, would not be very efficient in retaining nutrients within the plant-soil system during the initial stages of reclamation.

6.3 OIL SANDS MINESPOIL

i) Surface mining of the Oil Sands results in a minespoil which is almost pure sand devoid of nutrients and possessing a low CEC and poor water holding capacity. The pH of the tailing sand approaches neutrality one to two years after exposure to the elements.

ii) Amendation of the tailing sand with fertilizer significantly improved the NPK status of the sand, but many of these nutrients had leached out of the rooting zone by the end of the first growing season.

iii) The fertility status of the sand was greatly improved by the addition of sewage sludge. The fertility of the sludge amended sand appeared to be maintained over the 3 year term of the study.

iv) Application of peat to the tailing sand increased the N status of the soil, but available P was found to be potentially limiting.

v) As was observed for plants grown in the subalpine spoil, sewage sludge promoted the greatest shoot production by all four plant species (slender wheatgrass, sainfoin, jack pine and bearberry) over the term of the study.

vi) Fertilizer amendation was observed to be ineffective in stimulating plant growth presumably as a result of rapid loss of nutrients from the sand before plant uptake could occur. Slender wheatgrass performed best in the fertilized sand presumably because of its rapid growth rate and associated rapid immobilization of nutrients.

vii) The lack of extractable P in the peat is one factor which may have inhibited the primary production potential of all the test plant species. The slow growing bearberry performed best in the peat treated sand followed by jack pine and sainfoin. Some mineralization of bound P may have occurred in the peat after the first growing season.

viii) The growth pattern of slender wheatgrass was similar to that observed for slender wheatgrass grown in the subalpine spoil, suggesting this species is a short-lived perennial suitable for rapid cover of a site.

ix) Bearberry was highly susceptible to winter kill particularly in the fertilizer and sewage sludge treated tailing sand.

x) Foliage analysis of the slender wheatgrass and jack pine demonstrated that sewage sludge was the best amendment for providing N and P. Peat also supplied adequate quantities of N, but the P concentration in the jack pine needles was not significantly different from that observed for trees in the untreated sand, suggesting P in the peat was limiting to plant growth.

xi) The %N in the foliage of sainfoin was not influenced by amendation. Nitrogen was either supplied by the amendment or by N_2 fixation.

xii) Heavy metals (Cu, Zn, Fe) were not concentrated in the foliage of slender wheatgrass, sainfoin and jack pine after one growing season in the sewage sludge treated sand in comparison with foliage metal levels of plants grown in untreated sand.

xiii) The P concentrations recorded for the jack pine needles were highly correlated with soil P levels. This relationship did not hold for the slender wheatgrass and sainfoin. Heavy metal (Fe, Cu, Zn) concentrations in the soil were not strongly correlated with metal levels recorded in the foliage of slender wheatgrass, sainfoin and jack pine, suggesting that plant build up of toxic metals introduced in the sewage sludge did not occur over the short term.

7. RECOMMENDATIONS

1. In the case of all three minespoils (grassland, subalpine and extracted oil sands) sewage sludge amendation was highly effective in improving soil fertility and thereby stimulating plant growth. Sewage sludge also appeared to behave as a slow release fertilizer since plant growth was generally stimulated over the full term of the study (i.e. 3 years). Although sewage sludge, if readily available, is recommended for the reclamation of minesites, more research is required to study:

i) the effects of different levels of sewage sludge application to a minesoil on the germination, establishment and growth by the various plant species being considered in a revegetation program. Phytotoxic levels of NH_3 produced from drying anaerobically digested sewage sludge immediately after application may require that sludge amended soil be incubated for a certain length of time before seeding or planting.

ii) the rate of metal uptake by various plant species grown in sludge amended soil to determine if concentration of toxic metals by these plants will pose a problem over the long term. It should be kept in mind that release of heavy metals from sewage sludge and their subsequent availability and uptake by plants will vary with soil type, sludge characteristics and plant spp. For example, heavy metal availability to plants may be greater in a soil with a low CEC (such as the extracted oil sands) than in a soil with a high CEC (such as the grassland spoil).

iii) the rate of decomposition and associated nutrient release from sludge aggregates since this will determine the time span over which the fertility of a sludge amended soil can be maintained.

iv) the effects of sewage sludge on mycorrhizal development since toxic factors in the sludge can suppress mycorrhizal development in plants such as slender wheatgrass (see Zak et al., 1984).

2. Because the physical nature of the grassland minespoil (i.e. high clay and high Na levels) is the major factor impeding

plant growth, it is suggested that the SAR be monitored constantly when testing the effects of amendments such as gypsum.

3. More research is required on whether upward Na migration takes place when topsoil is placed over a highly sodic minespoil, and if so, at what rate does it move. Also, the effect of different plant species on Na migration from a sodic spoil into the overlying topsoil should be studied.

4. Since peat is notoriously lacking in plant available P, it should be supplemented with inorganic fertilizer (particularly phosphate) when used as an amendment in reclamation programs. Data on the optimum levels of fertilizer to be added to a particular plot to promote establishment and growth of various plant species (ranging from grasses to legumes to trees) are required.

5. Fertilization of minespoils which are often low in CEC and water holding capacity and therefore highly leachable, may result in a heavy loss of the added nutrients from the rooting zone. Further information is required on:

i) when to apply fertilizer so that plant uptake and immobilization of the nutrients is optimized. This will depend partly on whether the plant is slow or fast growing.

ii) the levels of fertilizer required by a plant to stimulate its growth, but not inhibit the formation of symbiotic relationships such as N_2 fixing nodules or mycorrhizae.

iii) the loss of fertilizer through leaching and runoff so that fertilization programs can be made more efficient.

iv) the effect of repeated fertilizer applications (particularly to a grass/herb mixture) on plant production, litter build-up, decomposition rates of the incoming litter and rates of nutrient release from the decomposing litter. Although shoot production may be stimulated by repeated fertilization, decomposition and nutrient release from the incoming plant litter may not be accelerated, resulting in the accumulation of a thick mat of dead vegetation which can become a fire hazard or a haven for rodents.

6. There is some evidence in the literature that difficulties may arise when attempts are made to establish woody

species in an area vegetated with grasses. Grasses compete with the woody plants for moisture and nutrients and may even produce allelopathic compounds which inhibit the growth of trees or shrubs. However, grasses and legumes form an essential component of a revegetation program since their rapid growth rate provides a quick cover for erosion control. Therefore if the establishment of woody plants in an already revegetated area is one of the aims of a reclamation program, more research is required to determine the effect of various grasses on the establishment and growth of various trees and shrubs.

7. Dinitrogen fixing legumes and shrubs can be important sources of N particularly in infertile minespoils which are highly leachable. Future research should examine the optimum nutrient conditions for promoting N_2 fixation by these plants and the effects of interseeding N_2 fixing legumes or shrubs on the growth of N nutrition of woody plants such as jack pine.

8. This study has dealt with the effects of individual amendments (applied to each of three minespoils) on the establishment and growth of individual plant species. More research is required to establish the effects of various amendments (either singly or in combination with other amendments) on the growth and competition of plants placed in groups (e.g. grass/legume; legume/tree; grass/tree) or planted as a mixture.

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Volume

2

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Volume II - Mycorrhizae

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LAND CONSERVATION AND
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Reclamation Research
Technical Advisory Committee

GREENHOUSE POT STUDIES DEALING WITH AMENDATION OF OIL SAND TAILINGS: EFFECTS OF PEAT, SEWAGE SLUDGE AND FERTILIZER ON PLANT GROWTH, MYCORRHIZAE AND MICROBIAL ACTIVITY

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ABSTRACT

Carex and feather moss peats from northern Alberta were used as growth media for jack pine and slender wheatgrass and their microbiological properties assessed. Differences between the two peats were minimal. Following this assessment, mixtures of the two peats were used to amend oil sand tailings (55% peat, V/V) and either mineral fertilizers or sewage sludge were added at different rates. Slender wheatgrass and jack pine were grown in the greenhouse in the various growth media, and plant growth, microbial activity and mycorrhizal development were measured. Growth of both species was poor without the addition of either fertilizer or sewage. Fertilization up to the equivalent of 112:49:72 kg N:P:K ha^{-1} substantially increased shoot and root growth of slender wheatgrass. Shoot growth of jack pine reached a maximum at the 28:12:18 kg ha^{-1} rate, and pine roots weights were depressed when more than 56:24:36 kg ha^{-1} were applied. Maximum shoot growth of slender wheatgrass and jack pine occurred with the equivalent of 46 and 23 mT ha^{-1} sewage respectively. The addition of 92 mT ha^{-1} strongly depressed the growth of jack pine while slender wheatgrass was unaffected. In the absence of peat, slender wheatgrass was much more sensitive to sewage. VA mycorrhizal inoculum in the peat was sparse and high levels of fertilizer or the lowest level of sewage completely inhibited VA mycorrhizal infection. Ectomycorrhizal inoculum was abundant but infections were nil when more than 56:24:36 kg N:P:K ha^{-1} was applied and very strongly reduced when 23 mT ha^{-1} or more of sewage was applied. In the presence of slender wheatgrass, fertilizer increased microbial biomass but had no effect when no plants were present, i.e. fertilizer did not affect the decomposition of peat. Microbial activity (CO_2 efflux) was unaffected by the addition of fertilizer but decomposition of grass litter was reduced by high rates of fertilizer. When sewage was added to the growing medium, microbial activity and microbial biomass were increased and the decomposition potential was decreased. The presence of the fibrous rooted slender wheatgrass consistently inhibited the decay of grass litter as compared to unplanted systems.

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1. GENERAL INTRODUCTION

It is clear that in order to establish vegetation and soil microbial processes in mine tailings, it is necessary to add ameliorating materials. The particular types and amounts of amendments used will be dependent on the tailings characteristics, the availability of amendments, economic considerations and the chemical and biological properties of the amendments. In a previous field study, amendments were added in single, individual applications and plant growth (Visser *et al*., 1984a), microbial relations (Visser *et al*., 1984b) and mycorrhizal development (Danielson *et al*., 1984; Zak *et al*., 1984) were monitored for three to four growing seasons. From this study and from information garnered from operational procedures it was apparent that certain factors relating to high nutrient additions and to rates and combinations of amendments were worthy of careful study under more controlled conditions.

In the studies cited above, sewage sludge proved to be a highly effective amendment in promoting plant growth over a moderately long period of time. However, sewage sludge had adverse effects on some mycorrhizal relationships and on symbiotic N_2 fixation while increasing the rate of cellulose decomposition. How different application rates of sewage sludge or combinations of sludge with a recalcitrant source of organic matter affects these processes and plant growth is unknown.

Mineral fertilizers are easily applied to spoils but unless slow release types are used, repeated applications may be required to maintain satisfactory plant growth. A single application of fertilizer in the field study stimulated plant growth but a considerable portion of the nutrients were lost through leaching during the first growing season. High concentrations of fertilizers may be applied to partially counteract the leaching losses but such levels may inhibit formation of mycorrhizae and waste expensive fertilizers. It seems more reasonable to use low, noninhibitory fertilizer levels and aim at minimizing leaching losses by incorporating adsorptive amendments and maximizing root and mycorrhizal development.

In the field study, peat was a generally satisfactory organic amendment especially for woody plants. The primary deficiency of peat was the low level of available P which apparently limited the growth of some fibrous rooted species. It would appear that the combination of peat with a source of available P would result in a superior growth medium. However, rates of mineral P additions should not be so high as to inhibit mycorrhizal development or the growth of plants adapted to low nutrient regimes.

In view of the possible interactions among amendment combinations and possibilities of producing a planting medium with chemical and biological properties superior to single-amendment media a series of greenhouse pot studies were conducted. The intention was to supplement the data collected during the field study as well as to provide specific information applicable to future field studies on oil sand reclamation.

1.1. LITERATURE CITED

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2. MICROBIAL ACTIVITY, MYCORRHIZAL INFECTION AND PLANT GROWTH IN **CAREX** AND FEATHER MOSS PEATS FROM NORTHERN ALBERTA

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2.1. ABSTRACT

Slender wheatgrass and jack pine were grown in *Carex* and feather moss peats and a 1:1 mixture of the two peats in pots in the greenhouse. The three peat types were added to oil sand tailings at rates of 30, 55 and 80% (v/v) and all treatments were fertilized at a rate of 112:49:72 kg NPK ha^{-1}. Plant growth was only affected to a minor degree by either the type or quantity of peat. VA mycorrhizal infections were not detected and ectomycorrhizae were infrequent. Respiration was the same for all types but decomposition potential and microbial biomass were least in the *Carex* peat. The presence of slender wheatgrass increased respiration and microbial biomass and decreased the decomposition of roots in litter bags. It is recommended that, for future experiments, a mixture of peats be applied at a rate of 55% (v/v).

2.2. INTRODUCTION

Two basic types of peat deposits occur in northern Alberta, moss peat and Carex peat (Turchenek and Lindsay, 1978). Moss peats develop from sphagnum or feather mosses in bogs which are usually treed with black spruce and tamarack as well as having a ground cover of ericaceous shrubs. Carex peats originate in fens dominated by Carex species in open peat lands with few woody plants (Turchenek and Lindsay, 1978).

Differences in chemical and biological properties of these two peat types may affect their usefulness as amendments on extracted oil sand tailings. Vegetation on the undisturbed peat, as well as depth of the deposits, will affect the density and quality of mycorrhizal inoculum available for both VA and ectomycorrhizal hosts which may be planted on the extracted oil sands and overburden stockpiles. Microbial biomass, decomposition potential and nutrient levels in peat amendments may all affect nutrient release and retention and thus the economies of establishing and maintaining the necessary vegetation cover on areas in need of reclamation. This present study was conducted to determine if peat type should be considered in both experimental and operational reclamation situations.

The objectives of the study were: (1) to assess the effects of peat type on the growth of slender wheatgrass and jack pine in pots under greenhouse conditions, (2) to determine the effects of adding different amounts of peat to sand-silt-clay mixtures on plant growth and microbial activity, and (3) to determine how the presence or absence of plants in the peat-mineral mixtures affects microbial processes. Data obtained in this study will be used in planning additional greenhouse pot studies and will provide information for field studies.

2.3. MATERIALS AND METHODS

A sample of feather moss peat was obtained from a drained black spruce-tamarack bog on the Syncrude lease near Fort McMurray, Alberta. The sample of Carex peat was obtained from the Suncor site in the same vicinity. These peats plus a silt-clay mineral fraction from a Syncrude overburden deposit were mixed with extracted oil sand tailings from the Syncrude dyke to produce planting mixtures. The nine planting mixtures were composed of Carex or feather moss peat or a 1:1 (v/v) mixture of the two peats. These three peat types were then mixed into the mineral fractions to give final peat contents of 30, 55 and 80% (v/v). The mineral mixtures used were composed of tailing sand and silt-clay overburden at a ratio of 19:1 for the 30 and 55% peat applications and 4:1 for the 80% peat application. These mixtures of peat and mineral fractions are currently being recommended for the reclamation of oil sands tailings (P. Sims, pers. comm.).

The same amount of fertilizer was added to all nine treatments. The equivalent of 493 kg/ha of 23:23:0 and 146 kg/ha of 0:0:62 was used as these applications have been used in other reclamation studies (Visser et al., 1984). This amounted to 0.44 g of 23:23:0 and 0.13 g 0:0:62 per pot. The fertilizer was mixed into the planting mixtures and placed in 12.5 cm diameter pots. Three slender wheatgrass seeds or three pregerminated jack pine seeds were planted in two-thirds of the pots. The remaining pots were left unplanted but otherwise treated like the planted pots. Five replicate pots of each treatment (9 planting mixtures, 3 planting treatments) were prepared. In addition, 15 pots were prepared with autoclaved 80% mixtures and planted with jack pine to detect airborne ectomycorrhizal inoculum. The greenhouse temperatures varied from 18 to 25°C and supplemental light was used to give a 15 h day length with a minimum of 3.5 klx.

Prior to planting, each of the four components of the planting mixtures and each of the 9 planting mixtures were analyzed for key chemical properties. Organic matter content was estimated by determining loss on ignition at 400°C for 24 h, and pH was determined in water. Total N, NO_3-N and extractable P was determined according to McKeague (1976).

The slender wheatgrass was harvested after 10 wk and the jack pine after 13 wk. The number of tillers of each slender wheatgrass plant were counted, the grain removed and grain and shoots dried at 80°C and weighed. Roots were washed free of planting mixture, subsampled for VA mycorrhizal evaluations, dried and weighed. Jack pine shoots were dried and weighed and the roots reserved for ectomycorrhizal evaluations prior to drying.

For VA mycorrhizal evaluations, slender wheatgrass roots were cleared and stained with .01% trypan blue (Phillips and Hayman, 1970). Roots were scanned for infections but not quantitatively evaluated. All the short roots of jack pine were rated for mycorrhizal infection in the 30 and 80% rate treatments. The number of short roots ranged from 41 to 611 per pot. Roots were examined at 12X and to confirm infections, whole mounts were examined with a 40X brightfield objective.

Following harvesting of the plants, basal respiration (CO_2 efflux) in each planting mixture was determined on samples placed in tubes connected to an "Ultragas 3" CO_2 analyzer (Wosthoff Company, Bochum, Germany). Microbial biomass C was determined on the same samples using the method of Anderson and Domsch (1978).

Decomposition potential of each planting mixture was estimated by determining the weight loss of slender wheatgrass roots in litter bags. One preweighed nylon litter bag (1.0 mm mesh) containing about 0.25 g of roots was buried in each pot prior to planting the plant species. When the plants were harvested, the litter bags were removed, cleaned, washed for 15 sec on each side, dried at 35°C and weighed.

2.4. RESULTS

The two peats were similar in pH, total N content and extractable P (Table 1). However, the Carex peat contained much higher levels of NO_3-N than the feather moss peat. The two mineral fractions were both low in organic matter and N. The pH of the planting mixtures were all similar and in a favorable range (Table 2).

Neither peat type nor rate of application affected the shoot growth of slender wheatgrass (Table 3). Root growth was uniformly superior with the 55% application. The number of tillers produced was least with plants that were grown in pots with 80% feather moss peat or the mixture (Table 4). Seed production was significantly less in the 30% Carex and feather moss peat treatments than in any of the other treatments.

Both shoot and root growth of jack pine seedlings were greater in planting mixtures containing Carex peat than with feather moss peat or a mixture of the two peats (Table 5). The rate of peat application also affected shoot and root growth with the 55% rate, allowing superior plant growth. About 10% of the pine seedlings in the feather moss and mixture treatments died of a damping off disease and attempts to replant the seedlings failed as new seedlings also died.

Basal respiration did not differ significantly among the three peats either in the absence or presence of plants (Table 6). In pots containing 30% peat, respiration was not affected by the presence of plants, whereas when 80% peat was used CO_2 efflux was significantly greater in the planting mixture with slender wheatgrass than with jack pine or in the unplanted mixture. Respiration was about 3 to 5-fold greater with the 80% treatment than with the 30% treatment.

Increasing the amount of peat from 30 to 80%, significantly increased the microbial biomass C 4 to 6 times (Table 7). When 80% peat was used, there was significantly less microbial biomass C in the Carex peat than in the feather moss peat or the mixture. At

Table 1. Characteristics of two peats, extracted oil sands and mineral amendment used to prepare planting mixtures.

	pH	Loss on ignition (%)	Total N(%)	NO_3-N $\mu g/g^{-1}$	Extractable P $\mu g/g^{-1}$
Feather moss peat	5.6	81	2.2	40	4.8
Carex peat	5.2	89	2.3	278	2.6
Silt-clay	7.4	5	.1	.5	2.6
Extracted oil sands	7.3	1	.01	1	1.6

Table 2. Amounts of peat added to pots and characteristics of the final mixtures prior to planting.

Peat type	% peat (v/v)	Field equivalent of dry peat (mT ha^{-1})	Final Mixture pH	% H_2O at 1/3 bar (wet wt.)	Loss on ignition (%)
Carex	30	25	6.0	12	3
Feather moss	30	21	5.9	12	4
Mixture	30	25	6.1	11	3
Carex	55	48	5.9	23	6
Feather moss	55	38	5.8	28	8
Mixture	55	45	6.0	21	10
Carex	80	69	6.1	49	21
Feather moss	80	54	5.8	56	30
Mixture	80	66	5.5	55	22

Table 3. Effect of peat type and rate of application on shoot and root growth of slender wheatgrass.

Rate of peat application (v/v)	Type of peat Carex	 Feather moss	 Mixture	Row means
		Shoot weight (g)		
30%	3.6^a	4.2^a	4.3^a	--
55%	4.3^a	4.1^a	4.7^a	--
80%	4.2^a	4.0^a	3.8^a	--
		Root weight (g)		
30%	1.0	1.4	1.6	1.4^a
55%	1.9	1.7	1.7	1.8^b
80%	1.2	1.1	1.2	1.2^a
Column means	1.4^a	1.4^a	1.5^a	

Data analyzed by two-way ANOVA and differences tested by Scheffé pairwise comparisons. Values for shoot weights followed by the same letter not significantly different (p = .05). Values within root mean set or peat mean set followed by same letter not significantly different (p = .05).

Table 4. Effect of peat type and rate of application on tillering and seed production of slender wheatgrass.

	Type of Peat		
Rate of peat application (v/v)	Carex	Feather moss	Mixture
	Number of tillers per plant		
30%	10^{bc}	10^{bc}	12^{c}
55%	10^{bc}	9^{ab}	9^{ab}
80%	8^{ab}	7^{a}	7^{a}
	Seed weight per plant (mg)		
30%	22^{a}	35^{ab}	54^{abcd}
55%	73^{bcd}	76^{bcd}	43^{abc}
80%	88^{d}	80^{cd}	74^{bcd}

Data analyzed by two-way ANOVA and differences among means tested by Scheffé pairwise comparisons. Values followed by the same letter are not significantly different (p = .05).

Table 5. Effect of peat type and rate of application on shoot and root growth of jack pine.

	Type of peat			
	Carex	Feather moss	Mixture	Row means
	Shoot weight (mg)			
30%	90	50	70	70^a
55%	170	100	70	110^b
80%	110	70	60	80^a
Column means	120^b	70^a	70^a	
	Root weight (mg)			
30%	19	13	14	15^a
55%	31	24	21	25^b
80%	21	12	11	15^a
Column means	24^b	16^a	16^a	

Data analyzed by two-way ANOVA and differences among means tested by Scheffé pairwise comparisons. Values within each rate mean set or peat mean set followed by the same letter are not significantly different (P = .05).

Table 6. Effect of peat type and rate of application on basal respiration ($CO_2\uparrow$) of planting mixtures in pots planted with slender wheatgrass or jack pine or left unplanted.

Peat type	Application rate (%)	No plant	Slender Wheatgrass	Jack pine
		Respiration (μl $CO_2\uparrow$ 100 g^{-1} of planting mixture h^{-1})		
Carex	30	46^{ab}	54^{ab}	53^{ab}
Feather moss	30	40^{ab}	82^{bcd}	57^{abc}
Mixture	30	34^{b}	67^{abc}	53^{ab}
Carex	80	117^{cde}	343^{fg}	199^{ef}
Feather moss	80	184^{ef}	356^{fg}	146^{de}
Mixture	80	244^{efg}	423^{g}	182^{ef}

Data could not be analyzed by using a three-way ANOVA due to significant interactions. Data in each planting treatment analyzed with a separate two-way ANOVA and differences among means tested by Scheffé pairwise comparisons. Values in each column followed by the same letter do not differ significantly ($p = .05$). Data was ln Y transformed prior to analysis.

Table 7. Microbial biomass C (mg C 100 g^{-1} mixture) in two types of peat and a mixture of the two peats and either planted with slender wheatgrass or jack pine or left unplanted. Data presented as overall treatment means[1].

	Rate of peat application	
Treatment	30%	80%
Carex peat	5.4^{a}	21.9^{a}
Feather moss peat	5.6^{a}	32.8^{c}
Mixture of peats	5.9^{a}	37.0^{c}

	Rate of peat application	
	30%	80%
No plants	4.0^{a}	24.0^{c}
Slender wheatgrass	10.0^{b}	55.7^{d}
Jack pine	4.4^{a}	19.9^{c}

	No plant	Slender Wheatgrass	Jack pine
Carex peat	7.0^{a}	20.9^{c}	8.8^{ab}
Feather moss peat	11.5^{b}	22.3^{c}	9.7^{ab}
Mixture of peats	11.8^{b}	28.3^{c}	9.5^{ab}

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[1] Data analyzed by 3-way ANOVA and no significant 3-way interactions were present. Each pair of parameters analysed by 2-way ANOVA and differences among overall treatment means compared with Scheffé pairwise comparisons. Values within each of the three sets not significantly different (p = .05) if followed by the same letter. Data ln Y transformed for analysis, values geometric means.

both the low and high rates of application of all peat types, the greatest amount of microbial biomass C was found in the planting mixtures in which slender wheatgrass had been grown. The presence of jack pine did not affect the amount of microbial biomass C. In the absence of plants the microbial biomass C was least in the Carex peat treatment but when plants were present, peat treatment had no effect.

The presence of slender wheatgrass roots significantly inhibited root decomposition in all three peat treatments (Table 8). The type of peat used in the planting mixtures affected the rate of decomposition, with the roots in the litter bags buried in the Carex treatment decomposing slower than those in the feather moss treatments. However all differences were relatively small.

Observations on the VA mycorrhizal status of slender wheatgrass roots indicated that there were no infections in any treatment. Mycorrhizal development of jack pine was very low and sporadic. No infection was detected in the 30% application rate in any of the three peat treatments nor in the autoclaved treatments. In the 80% Carex peat treatment, one pot contained heavily infected roots but the symbiont was Thelephora terrestris Ehrhard ex Fr. and it is assumed that the infection was due to airborne contamination. One other pot in the 80% carex treatment had infected roots but only 2% of total short roots were mycorrhizal.

In the 80% feather moss and mixture treatments 2% of the short roots were mycorrhizal and 8 of the 10 trees had some infection. All of the symbionts appeared to be Basidiomycetes except for one occurrence of the I-type Ascomycete.

Table 8. Decomposition of slender wheatgrass roots in litter bags buried in three types of peat planted with slender wheatgrass or jack pine or left unplanted.

	Peat type			Row means
	Carex	Feather moss	Mixture	
Plant treatment	Percent weight remaining			
No plants	61	49	52	54^{a}
Slender wheatgrass	68	64	67	66^{b}
Jack pine	57	46	47	50^{a}
Column means	62^{b}	53^{a}	55^{ab}	

Data analyzed with a two-way ANOVA and differences tested by Scheffé pairwise comparisons. Values within planting mean set or peat mean set followed by the same letter not significantly different (p = .05).

2.5. DISCUSSION

Although the two peats were of quite different origins, in the short term greenhouse study conducted here, differences in plant growth responses were minor. Differences may have been minimized by the application of fertilizer to all treatments although the growth of jack pine was 70% greater with the Carex peat than with the feather moss peat. Regardless, the jack pine seedlings were very small in all treatments considering the amount of fertilizer applied (Part 3) and differences should not be overemphasized. The feather moss peat contained damping off organisms which may have contributed to the poor growth of jack pine in this treatment. VA mycorrhizae were absent and ectomycorrhizae were poorly developed in both peat types. It appeared that ectomycorrhial inoculum was more abundant in the feather moss peat than in the sedge peat, but infections were too sporadic to make definite conclusions. The low level of ectomycorrhizal infection and the lack of VA infection in this experiment was due to suppression by the fertilizer applied (Part 3).

Basal respiration did not differ among the peat types but decomposition rates and microbial biomass were both least in the Carex planting mixture. The lack of differences in CO_2 efflux would suggest that both peats were of similar stability and would persist for equal lengths of time if used to amend tailings sand.

Growing slender wheatgrass in the mixtures enhanced respiration and increased the microbial biomass present. However, decomposition of introduced root material was inhibited by the presence of slender wheatgrass. Jack pine did not affect these factors, probably because of the much smaller root systems.

In general, no peat type or rate of application was clearly superior to the others. For greenhouse studies the medium (55%) rate of application is a reasonable choice. Although the mycorrhizal factor could not be evaluated, the use of a mixture of the two peat types would offer the best opportunity for including a variety of inoculum. Thus it is recommended that a 55% mixture of the carex and feather moss peats be used in future experiments.

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3. MICROBIAL ACTIVITY, MYCORRHIZAL INFECTION AND PLANT GROWTH RESPONSES TO FERTILIZATION OF PEAT AMENDED OIL SAND TAILINGS

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3.1. ABSTRACT

Extracted oil sands were amended with 55% (v/v) peat and fertilized with a fertilizer equivalent to 112:49:72 kg NPK ha^{-1} and 1/2, 1/4, 1/8 and 0 fractions of that rate. Slender wheatgrass and jack pine were grown in the greenhouse for 10 and 15 wk respectively in each of the five fertilizer regimes. Root and shoot weight of slender wheatgrass increased with each incremental addition of fertilizer whereas growth of jack pine reached a maximum with 1/4 of the highest rate. Further additions reduced root growth of jack pine while shoot growth remained constant. Mycorrhizal formation by jack pine also decreased substantially above the 1/4 level. Less than 2% of the roots of slender wheatgrass were infected in the control and 1/8 treatment and none with the high fertilizer. Respiration and microbial biomass were unaffected by fertilizer additions in the absence of plants but decomposition potential was reduced with the highest level of fertilizer. In the presence of slender wheatgrass, moderate levels of fertilizer significantly increased microbial biomass while the high fertilizer application resulted in a level of microbial biomass similar to the control. Decomposition potential was less when slender wheatgrass was present than in unplanted pots.

3.2. INTRODUCTION

Fertilizer is the most common amendment used in operational reclamation procedures as it is felt that the addition of nutrients is a prerequisite for the establishment of plants. However, fertilizer may have less favorable effects on other components of the system, in particular, below ground processes. Preliminary observations suggested that normal rates of fertilization can depress microbial activity (Visser et al., 1984) which may affect decomposition of litter and nutrient cycling.

The sensitivity of mycorrhizal symbioses to P is well known and fertilization may substantially inhibit mycorrhiza formation (Gerdemann, 1975). As pointed out by Rhodes (1980), the use of a P fertilizer may cause a suppression of VA mycorrhizae and, although P is abundant, micronutrient deficiencies may develop as the soil is inefficiently exploited by nonmycorrhizal root systems. On the other hand, when P levels are very low, mycorrhizal formation may also be inhibited (Mosse et al., 1981). Thus fertilization may either positively or negatively affect mycorrhizae and the specific effect is difficult to predict as both host and symbiont are determining factors (Mosse et al., 1981). Presumably, symbionts are adapted to nutritional conditions of the soil in which they exist (or more precisely, the nutritional status including carbohydrates and P levels of the host roots), but once an area has been severely disturbed, the nutritional status of the substrate is changed. Also, if nonindigenous hosts are also introduced, the symbionts indigenous to the undisturbed habitat may be less well adapted than species that could be introduced. It is of interest then, to determine how indigenous symbionts react to reclamation practices such as fertilization.

As concern has been expressed over the loss of C from peat amendments and the possible deterioration of reclaimed spoils (see discussion in Ziemkiewicz et al., 1980) it is of interest to determine the effects of nutrient enrichment on the rate of peat decomposition. By manipulation of fertilizer levels, it may be possible to use this valuable resource more economically and efficiently.

The objectives of this greenhouse pot experiment using peat amended oil sand tailings were to determine (1) the effects of different levels of application of NPK fertilizer on primary production, (2) the effects of fertilizer on the rates of decomposition of a standard introduced substrate (decomposition potential), (3) the effect of fertilizer and plants on the CO_2 efflux from the planting mixture, (4) the effect of fertilizer and plants on microbial biomass and (5) the effect of different amounts of fertilizer on VA and ectomycorrhizal development.

3.3. MATERIALS AND METHODS

The experimental materials used were similar to those used in the previous experiment (Part 2). One substrate mixture was used for all the treatments, a 1:1 mixture of Carex peat and feather moss peat plus 45% (v/v) mineral additive. The mineral portion was a 19:1 (v/v) mixture of extracted tailings sand and silt-clay overburden. Fertilizer was added at a top rate of 493 kg/ha of 23:23:0 and 146 kg/ha of 0:0:62 (field equivalent) and three fractions thereof plus a nonfertilized treatment. The amount of fertilizer added per pot and the treatments were: 1x = .44 g 23:23:0 + .13 g 0:0:62 and 1/2, 1/4 and 1/8 of these amounts. Each of these fertilizer combinations and a control (zero fertilizer) were mixed with the planting mixture and placed in 15 12.5 cm diameter pots per fertilizer level. Five pots of each fertilizer level were planted with three slender wheatgrass seeds per pot, five pots with three pregerminated jack pine seeds per pot and the remaining five pots were left unplanted. Thus the final treatments included five fertilizer rates and three planting treatments. In addition, five pots were filled with unfertilized planting mixture, autoclaved and planted with jack pine seeds to detect air-borne ectomycorrhizal inoculum.

The slender wheatgrass was harvested after 10 wk and the jack pine after 15 wk. Tillers of slender wheatgrass were counted, the seed removed and leaves and seed dried at 80°C and weighed. Roots were washed, subsampled for VA mycorrhizal evaluations, and dried and weighed. Jack pine shoots were dried and weighed and the roots washed and evaluated for mycorrhizal infection prior to determining dry weights.

Slender wheatgrass roots in the control, 1/8x and 1x treatments were cleared and stained with .01% trypan blue (Phillips and Hayman, 1970) and evaluated according to Zak and Parkinson (1983). Jack pine roots in all treatments were cut into 2-3 cm segments and the segments randomly sampled until 300 short roots had been rated for infection (Part 2).

Following harvesting of plants, the basal respiration of the spoil mixtures was determined with an "Ultragas 3" CO_2 analyzer

(Wosthoff Company, Bochum, Germany). Microbial biomass C was determined on the same samples using the glucose stimulated respiration method of Anderson and Domsch (1978).

Decomposition potential was estimated by placing approximately .25 g of slender wheatgrass leaves in 1.0 mm mesh nylon litter bags and burying one preweighed bag per pot prior to planting the test plants. When the plants were harvested, the litter bags were retrieved, cleaned, dried at 35°C and weighed.

3.4. RESULTS

Slender wheatgrass shoots and roots showed a clear and consistent response to increasing levels of NPK mineral fertilizer (Table 1). Seed production as well as the number of tillers per plant also responded to the fertilizer applications. Roots and shoots responded relatively uniformly as indicated by the shoot/root ratios. In the absence of any fertilizer, the peat-tailing sand mixture supported little growth of the grass and no reproductive capability as no flowers were formed. In all the other treatments, flowers were formed 7 wk after planting.

Jack pine shoot and root growth reached maximum weights with the 1/8x to 1/4x application rates (Table 2). Shoot growth did not change when more than 1/4x fertilizer was added. However, root growth was depressed by rates exceeding 1/4x such that shoot/root ratios increased dramatically. The reduction in root weights at high fertilizer levels also resulted in a decrease in the number of short (feeder) roots. Autoclaving the planting mixture did not significantly affect the growth of jack pine.

The amount of VA infection was very low in the control (zero fertilizer treatment), occurring only in one pot (Table 3). In the 1/8x treatment, infection occurred in four of the five pots but only small portions of the root systems were mycorrhizal. All infections were caused by a coarse endophyte. Total root lengths were markedly stimulated by fertilization although the rate of application did not result in different lengths.

Rates of ectomycorrhizal infections of jack pine were not significantly affected by additions of fertilizer except with the 1x treatment (Table 4). The major symbionts were unknown Basidiomycetes. It was not possible to determine if this group included one or more taxa. It appeared that the second most common group, termed white dichotomous, was sensitive to fertilizer additions as it was not encountered in the fertilizer treatments. The only two types that could be recognized were the Rhizopogon-like symbiont and the I-type Ascomycete, both of which were rare. The

Table 1. Growth of slender wheatgrass in peat amended with different amounts of fertilizer (1x equivalent to 112:49:72 kg N:P:K ha^{-1}).

Parameter	Fertilizer level				
	0	1/8x	1/4x	1/2x	1x
Shoot weight (g)[1]	$.34^a$	1.78^b	2.36^c	3.54^d	4.26^e
Root weight (g)[1]	$.27^a$	1.28^b	1.56^c	2.02^d	2.27^e
Seed weight (g)[2]	0	$.09^a$	$.39^b$	$.77^c$	$.74^c$
Shoot/root ratio	1.3	1.4	1.5	1.8	1.9
Tillers $plant^{-1}$	2.6^a	4.9^b	6.2^c	7.6^d	9.7^e

[1] ln Y transformation required.

[2] ln (Y + 1) transformation required.

Data analyzed by one-way ANOVA and differences tested with Scheffé pairwise comparisons. Values in each row followed by the same letter not significantly different (p = .05).

Table 2. Growth of jack pine in peat amended with different amounts of fertilizer (1x equivalent to 112:49:72 kg N:P:K ha^{-1}).

	Fertilizer level					Auto-
Parameter	0	1/8x	1/4x	1/2x	1x	claved
Shoot weight (mg)	113^{a}	271^{bc}	384^{c}	398^{c}	378^{c}	185^{ab}
Root weight (mg)	74^{ab}	153^{c}	187^{c}	113^{abc}	69^{a}	130^{b}
Shoot/root ratio	1.5^{ab}	1.8^{ab}	2.1^{b}	3.5^{c}	5.4^{d}	1.4^{a}
Short roots plant^{-1}	340^{ab}	415^{abc}	661^{c}	354^{abc}	231^{a}	603^{bc}

Data ln Y transformed and analyzed by one-way ANOVA and differences tested with Scheffé pairwise comparisons. Values in each row followed by the same letter not significantly different ($p = .05$).

Table 3. VA mycorrhizal infection and root lengths of slender wheatgrass fertilized at two rates and unfertilized (1x equivalent to 112:49:72 kg NPK ha^{-1}).

	Control	1/8x Fertilizer	1x Fertilizer
Total root length ($m\ l^{-1}$)	92^a	489^b	443^b
Mycorrhizal root length ($m\ l^{-1}$)			
Total	1.9	7.9	0
With hyphae	1.7	6.3	0
With vesicles	.03	.06	0
With arbuscules	.2	1.6	0
Percent infection ($\bar{x} \pm$ SD)	1.6 ± 3.6	1.8 ± 2.0	0

Root length data ln Y transformed prior to analysis by one-way ANOVA and differences tested by Scheffé pairwise comparisons. Values followed by the same letter not significantly different ($p = .05$).

Table 4. Percent mycorrhizal infection of jack pine seedlings growing in peat with different levels of fertilizer (1x equivalent to 112:49:72 kg N:P:K ha^{-1}).

Infection	Fertilizer level									
	0		1/8x		1/4x		1/2x		1x	Autoclaved
	$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD		
Total	49^a	29	47^a	23	42^a	29	16^a	16	0	0
Unknown types	28	17	46	23	29	26	12	17	0	0
Hyaline basidiomycete	21	26	0	0	0	0	0	0	0	0
Rhizopogon-like	.3	.5	.4	.9	.3	.8	0	0	0	0
I-type ascomycete	0	0	1.1	2.4	12	27	4	8	0	0

Data analyzed by one-way ANOVA and differences tested by Scheffé pairwise comparisons. Values followed by the same letter do not differ significantly ($p = .05$).

Rhizopogon-type infected only 1-2% of the short roots of seedlings it colonized and the I-type infected 5 to 61% of the short roots. Roots in the 1x and autoclaved treatments were uninfected.

Basal respiration of mixtures planted with slender wheatgrass or jack pine did not differ from unplanted mixtures (Table 5). Fertilization had no effect on respiration of peat planted with slender wheatgrass. In peat planted with jack pine, respiration was significantly greater with the 1/8x fertilizer treatment than with the high application rate.

Ten and 15 wk after initiation of the experiment, microbial biomass C did not differ significantly among the unplanted treatments (Table 6). However, in peat planted with slender wheatgrass microbial biomass C was significantly higher in the 1/8x, 1/4x and 1/2x fertilizer treatments than in the control and the 1x treatment. The latter two treatments did not differ significantly from the unplanted peat. Fertilization did not affect amounts of microbial biomass C in peat planted with jack pine. Fertilization of slender wheatgrass resulted in increased amounts of microbial biomass C in all fertilized peats as compared with peats planted with jack pine although the jack pines were 5 wk older than the slender wheatgrass.

The presence of slender wheatgrass plants resulted in less decay of buried slender wheatgrass leaves than in unplanted peat (Table 7). Fertilization at the 1x level resulted in a decrease of decomposition potential in the peat planted with slender wheatgrass. Decomposition had apparently slowed considerably by 10 wk as there were no differences in weight remaining between 10 and 15 wk except in the 1x fertilizer treatment.

Table 5. Basal respiration of peat amended with different amounts of fertilizer and planted with slender wheatgrass, jack pine or left unplanted. Values are $\mu l\ CO_2 + 100\ g\ h^{-1}$ growing medium (1x equivalent to 112:49:72 kg NPK ha^{-1}).

	Fertilizer level					Row
Treatment	0	1/8x	1/4x	1/2x	1x	means
Control (10 wk)	117	96	96	106	96	102^{a}
Slender wheatgrass	115	124	112	121	121	119^{b}
Column means	116^{a}	110^{a}	104^{a}	114^{a}	109^{a}	
Control (15 wk)	96	112	128	112	96	109^{a}
Jack pine	102	140	115	96	64	103^{a}
Plant mean	99^{ab}	126^{b}	122^{ab}	104^{ab}	80^{a}	
Slender	115^{ab}	124^{ab}	112^{ab}	121^{ab}	121^{ab}	
Jack pine	102^{ab}	140^{b}	115^{ab}	96^{ab}	64^{a}	

Data analyzed by two-way ANOVA and differences tested using Scheffé pairwise comparisons. Values within each plant mean set or fertilized mean set followed by the same letter do not differ significantly ($p = .05$). For jack pine, slender wheatgrass comparisons, values followed by the same letter do not differ significantly ($p = .05$).

Table 6. Microbial biomass C in peat amended with different amounts of fertilizer and planted with slender wheatgrass or jack pine or left unplanted. Values are mg C 100 g h^{-1} growing medium (1x equivalent to 112:49:72 kg NPK ha^{-1}).

	Fertilizer level				
Treatment	0	1/8x	1/4x	1/2x	1x
Control (10 wk)	10.1^a	12.0^a	12.3^a	11.0^a	8.4^a
Slender wheatgrass	10.6^a	18.9^b	17.7^b	17.5^b	16.2^a
Control (15 wk)	7.4^{ab}	13.1^{ab}	13.8^b	10.6^{ab}	7.4^{ab}
Jack pine	9.3^{ab}	10.6^{ab}	9.3^{ab}	10.1^{ab}	7.8^a
Slender wheatgrass	10.6^a	18.9^b	17.7^b	17.5^b	16.2^b
Jack pine	9.3^a	10.6^a	9.3^a	10.1^a	7.8^a

Data analyzed by two-way ANOVA and differences tested by Scheffé pairwise comparisons. Values within each set followed by the same letter do not differ significantly ($p = .05$).

Table 7. Decomposition of slender wheatgrass leaves buried in peat amended with different amounts of fertilizer. Values are percent weight remaining (1x equivalent to 112:49:72 kg NPK ha^{-1}).

	Fertilizer level					Row
Treatment	0	1/8x	1/4x	1/2x	1x	means
No plants (10 wk)	17	16	14	17	27	18^a
Slender wheatgrass	16	20	25	24	34	24^b
Column mean	16^a	18^a	20^a	21^a	31^b	
No plants (15 wk)	15	18	14	14	15	15^a
Jack pine	17	14	14	11	15	14^a
Plant mean	16^a	15^a	12^a	14^a	16^a	
No plant (10 wk)	17	16	14	17	27	
No plant (15 wk)	15^a	18^a	14^a	14^a	15^a	

Data analyzed by two-way ANOVA and differences tested by Scheffé pairwise comparisons. Values within each plant mean or fertilizer mean followed by the same letter not significantly different ($p = .05$). For the time comparison, values followed by the same letter do not differ significantly ($p = .05$).

3.5. DISCUSSION

Fertilization with a mineral fertilizer resulted in quite different growth response patterns with the two plant species. Slender wheatgrass positively responded to all levels applied with a maximum 12-fold increase in shoot growth over growth in the unfertilized control. In contrast, jack pine increased a maximum of 3.5-fold and shoot growth did not increase above a rate equivalent to 28:12:18 kg NPK ha^{-1} (1/4x). It is unknown whether similar results would occur with a top dressing of fertilizer in contrast to the incorporation technique used here. Clearly, planting slender wheatgrass rather than jack pine on oil sand would result in a more rapid accumulation of nutrients in plant biomass and litter. In a sandy spoil this would result in a conservation of fertilizer nutrients that are subject to leaching losses. As decomposition (of buried leaves) was not affected by nutrient levels, it might appear that increased fertilization would lead to increased litter accumulation. However, this might not be true as substrate quality will change with soil nutrient levels and affect decomposition rates.

Of considerable interest was the strong depression of root growth of jack pine by high nutrient levels, a feature not shown in shoot growth. This depression of root growth could result in reduced ability to survive under stress conditions on amended oil sand tailings. High levels of fertilizer resulted in small root systems and plants with high shoot/root ratios. During drought conditions, large shoot/root ratios may result in water deficits due to large transpirational areas and small absorptive surfaces. This would be further accentuated as high fertilizer levels resulted in a complete suppression of mycorrhizal development. The absence of mycorrhizae has been shown to restrict water transport to roots due to the lack of rhizomorphic "pipelines" (Duddridge _et_ _al_., 1980). VA mycorrhizal inoculum was apparently quite low and even when plants became infected the infections were not extensive. This suggests that spread through the root system was slow as was observed for some ectomycorrhizal symbionts, especially the Rhizopogon-like symbiont. In order to obtain high levels of infection of VA hosts growing in materials such as used here, inoculum must be increased substantially. However, this would be futile if large amounts of fertilizer are used which would suppress infection.

Increasing the level of N, P and K generally did not affect basal respiration of the peat. This would infer that fertilization will not result in increased loss of peat-C and accelerate organic matter losses. A large portion of the peat is apparently composed of recalcitrant C compounds which decay very slowly even when N, P and moisture are abundant. However the presence of a fibrous rooted plant such as slender wheatgrass appeared to accelerate the loss of peat C. The increased CO_2 efflux from peat planted with slender wheatgrass as compared with unplanted peat could be due to a priming effect of the root exudates or it might be an artifact resulting from the inability to remove very fine root fragments. As the existence of a priming effect appears to be doubtful, the cause of increased CO_2 output remains to be determined.

In unplanted spoil, amounts of microbial biomass C was not affected by fertilization just as basal respiration was unaffected. However, when planted with slender wheatgrass, there was an increase in microbial biomass when moderate amounts of fertilizer were applied. It appears likely that the increased biomass was the result of utilization of root exudates by the rhizosphere and soil microflora (Visser et al., 1984). The amount of roots was very small with the slender wheatgrass control and all the jack pine treatments, resulting in small amounts of exudates available in the rhizosphere for microbial growth. The reduced amounts of microbial biomass at the higher fertilizer level was probably due to a fertilizer induced inhibition of microbial growth (Kowalenko et al., 1978). In all three pot systems, grass, pine and unplanted, there was a trend toward less microbial biomass as fertilizer levels increased above the 28:12:18 kg NPK ha^{-1} (1/4x) application rate. It might be expected that this would also result in a decrease in the amount of decomposition as was determined in the slender wheatgrass treatments. The apparent lack of effects with jack pine was probably due to the long burial period. By 10 wk, most of the leaves had been decomposed to the extent that only the most resistant and stable portions remained. Clearly organic matter (leaf litter) decomposition was inhibited both by the presence of a fibrous rooted plant and by high levels of mineral nutrients.

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4. EFFECTS OF PHOSPHORUS ADDED TO PEAT ON THE GROWTH OF ALSIKE CLOVER AND ON MICROBIAL PARAMETERS

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4.1. ABSTRACT

A P deficient peat was amended with 272 μg P g^{-1} peat, 544 μg P g^{-1} peat or left unamended. Pots were planted with alsike clover and grown for either 6 or 12 wk in the greenhouse. Added P increased the growth of shoots, roots and increased flower production. No differences in growth were apparent between the two levels of P. Decomposition of filter paper buried in the peat was enhanced with added P with the most rapid rate occurring in the high P peat. With increasing levels of P, respiration (CO_2 efflux) and microbial biomass C both decreased significantly. Phosphorus levels did not affect the number of nodules on the roots or their ability to fix N_2 (C_2H_2).

4.2. INTRODUCTION

Peat obtained from muskeg sites is a standard amendment used to improve the physical and chemical properties of extracted oil sands following the surface mining of bitumen bearing deposits in northern Alberta. Peat provides a long term source of nutrients for plant growth and improves the water holding properties of spoils, thus enhancing the development of maintenance-free soils that are required by provincial guidelines.

In a trial of a variety of amendments to determine their value in reestablishing biological activity in three contrasting types of minespoils (Visser *et al.*, 1984a, b; Danielson *et al.*, 1984; Zak *et al.*, 1984) a peat type obtained from a forested site was used to ameliorate the stressful conditions. The obvious characteristics of this peat were an unusually high content of NO_3-N, a moderate pH and a very low level of available P (Visser *et al.*, 1982a). As P appears to be a regulating factor in plant growth and microbial processes, and that P is often available in only small quantities in peat (Brady, 1974) it appeared to be worthwhile to investigate the effects of P availability in detail.

Over a three year period it was observed that the growth of alsike clover did not differ on a subalpine minespoil and on the same spoil heavily amended with the feather moss peat described above (Visser *et al.*, 1984a). A chemical analysis of the peat showed that the peat was very deficient in extractable P with less than 0.5 g extractable P per gram dry peat. It was therefore postulated that the lack of a growth response of alsike clover was due to the low level of available P. The objectives of this study were to determine (1) the effects of added P on the growth of alsike clover, and (2) the effect of added P on microbial biomass, basal respiration of the peat, decomposition potential and N_2 fixation potential of the clover.

4.3. MATERIAL AND METHODS

The peat was collected from unplanted pathways in the experimental tanks established 3 years previously (Visser et al., 1984a). The peat had been removed from a white spruce site 3 years previously and spread on a subalpine mine spoil. The peat was divided into 30 716g portions and moistened with water containing calcium phosphate, monobasic or super phosphate. Ten samples received no P, 10 received 272 μg P g^{-1} peat, and 10 received 544 μg P g^{-1} peat. The 272 μg P level was the same level that was applied in the field tank study (Visser et al., 1984a). After adding the P, the peat samples were left to equilibrate for 3 days in plastic bags. Then each bag of peat was emptied into a 12.5 cm plastic pot and a litter bag containing filter paper was placed in a vertical position in the middle of the pot. Each pot was planted with five 4 day old alsike clover seedlings and the pots were placed in the greenhouse in a complete random block design. The pots were kept at a constant moisture level by watering daily with distilled water to a specific weight.

After 6 and 12 wk, five pots of each P treatment were harvested and shoots, roots and flowers dried at 80°C and weighed. Filter papers were removed, washed, dried at 35°C and weighed to determine decomposition potential. Basal respiration (CO_2 efflux) and microbial biomass C of the root-free peat was determined using a Wosthoff CO_2 analyzer and the Anderson and Domsch (1978) technique. The number of root nodules was estimated by taking a subsample of the total root weight in each pot, spreading the roots in lactophenol on a slide, covering them with a coverslip and then counting the nodules under a dissecting microscope at 20x magnification. Prior to removing the plants from the peat, the peat plus plants minus the pot were placed in plastic containers, sealed and assessed for N_2 (C_2H_2) fixation capacity using the technique outlined by Visser et al. (1984b). Prior to injecting acetylene into the containers holding the plants, the plants were tested for natural ethylene production. Also unplanted peat was tested for asymbiotic N_2

(C_2H_2) fixation. No measureable ethylene was produced by the plants after one hour incubation. The low level of asymbiotic N_2 (C_2H_2) which was measured was subtracted from the N_2 (C_2H_2) fixation measured for each pot.

4.4. RESULTS AND DISCUSSION

Data on the effects of added P on alsike clover growth over the two sampling periods are presented in Table 1. These results show that there was an interactive effect of time and P level on shoot and root growth i.e., plant growth was not constant over the two sampling periods. When the treatment means were analyzed separately, it was observed that shoot, root and total plant weight were significantly greater in the P treated peat than in the control peat indicating that P was indeed limiting. However, there was no difference in plant weight produced at the low P level compared with that produced at the high P level demonstrating that the lower level of P would have been adequate over the short term. In all three treatments, there was a significant increase in shoot, root and total plant weight between 6 and 12 wk.

Results in Table 2 demonstrate that flower production was enhanced by the addition of P to the peat, but as for the roots and shoots, there was no difference in the weight of flowers produced at the two levels of P. Also the weight of flowers produced did not increase significantly from the first to the second sampling time. It is interesting to note that no flowers were produced by plants in the control pots, indicating the significance of adequate P availability to flowering and seed formation. The lack of seed production due to low P levels, would undoubtedly affect reseeding of alsike clover if the growing season was short (i.e. 3-4 months).

The decomposition of filter paper was enhanced by the addition of P to the peat (Table 3). This result indicates that the microorganisms involved in cellulose decomposition are, like the plants, limited in their activities by low P levels. As a result, low P levels in an amendment such as peat could markedly affect decomposition rates of incoming litter such as dead roots and shoots if these substrates were also low in available P.

The increased decomposition of filter paper in P-amended peat is interesting when the data in Table 4 are studied. These results show that with increasing levels of P, microbial biomass C

Table 1. Shoot weight, root weight and total plant weight of alsike clover grown in pots containing peat with and without added phosphorus.

	Time	Phosphorus added ($\mu g\ g^{-1}$ peat)		
	(wk)	0	272	544
			Dry weight (g pot^{-1})	
Shoot wt	6	0.14^{a}	1.16^{b}	.85^{b}
	12	1.02^{b}	3.33^{c}	3.46^{c}
Root wt	6	0.04^{a}	0.26^{b}	0.23^{b}
	12	0.42^{b}	0.56^{c}	0.51^{c}
Total plant wt	6	0.18^{a}	1.42^{b}	1.08^{b}
	12	1.44^{b}	3.89^{c}	3.97^{c}

Values in each data set were analyzed with a two-way ANOVA after ln transformation. Scheffé confidence intervals were computed for treatment means. Values within each data set followed by the same letter are not significantly different ($p = 0.05$).

Table 2. Flower production by alsike clover grown in pots containing peat with and without added phosphorus.

Time (wk)	Phosphorus added ($\mu g\ g^{-1}$ dry peat) 0	272	544	Row means
	Dry weight ($g\ pot^{-1}$)			
6	0	.089	.036	.063^a
12	0	.121	.094	.108^a
Column means	0^b	.105^a	.065^a	

By applying the Chi-square goodness of fit test for presence or absence of number of flowers produced pot^{-1}, the no phosphorus treatment plants produced a significantly lower number of flowers (no flowers) over each time period than the phosphorus treated plants. Hence the no phosphorus data was deleted from the two-way ANOVA.

Data for the phosphorus treatments were analyzed with a two-way ANOVA. Values in the amendment mean column or time mean row followed by the same letter do not differ significantly ($p = 0.05$).

Table 3. Decomposition of cellulosic filter paper in pots containing alsike clover grown in peat with and without added phosphorus.

	Sampling Time	Phosphorus level		
	(wk)	0	272	544
% dry wt remaining of filters	6	79.2[a]	45.5[b]	21.8[c]
% dry wt remaining of filters	12	3.7[a]	1.3[b]	0.7[b]

Data at each sampling time were analyzed using a one-way ANOVA and Scheffé confidence intervals. Values in each row followed by the same letter do not differ significantly ($p = 0.05$).

Table 4. Basal respiration and microbial biomass C in peat with and without added phosphate after removal of alsike clover roots.

	Time	Phosphorus level ($\mu g\ g^{-1}$ peat)			Row
	(wk)	0	272	544	means
Basal respiration ($\mu l\ CO_2 \uparrow 100\ g^{-1}$ dry peat hr^{-1})	6	98	54	35	62^a
	12	111	57	35	68^a
Column means		104^a	55^b	35^c	
Microbial biomass C (mg C 100 g^{-1} dry peat)	6	303	216	155	225^a
	12	302	228	168	232^a
Column means		302^a	222^b	161^c	

Data analyzed with a two-way ANOVA. Scheffé confidence intervals were computed for row and column means. Values within the time mean column or treatment mean row followed by the same letter do not differ significantly ($p = 0.05$).

and basal respiration decrease, an observation which is in direct opposition to the decomposition data.

Data in Table 5 demonstrates that there were no significant effects of P on the number of nodules which developed on the roots or on the N_2 fixing capacity of these nodules. Although many nodules were present on 6 wk old plants, they were not active as no acetylene was reduced. However, N_2 fixation did increase over the 6 to 12 wk sampling time indicating that the 6 wk nodules were either too immature to fix N_2 or their capacity to fix N_2 was retarded due to high levels of NO_3-N in the peat.

In conclusion, it appears that the low levels of extractable P in the peat used to amend the spoil material (Visser *et al.*, 1984a) markedly inhibited plant growth and flower production by alsike clover. Since peat is generally low in P (Brady, 1974), its usefulness as an amendment for the revegetation of disturbed soils might be limited unless additional P is applied prior to planting plant species such as alsike clover. If additional P were not applied, the plants would presumably be heavily dependent on mycorrhizal infection and development for supplying P necessary for growth and reproduction.

Table 5. Nodule numbers and N_2 (C_2H_2) fixation of alsike clover grown in pots containing peat with and without added phosphate.

	Time (wk)	Phosphorus level (μg/g dry peat)			Row means
		0	272	544	
Nodule number g^{-1} dry root	6	342	560	885	596[a]
	12	521	548	867	645[a]
Column means		432[a]	554[a]	867[a]	
N_2 fixation capacity (nmoles N_2 (C_2H_2) fixed g^{-1} dry root hr^{-1})	6	0	0	0	
	12	60.4[a]	34.8[a]	19.5[a]	

Values in the nodule number data set were analyzed with a two-way ANOVA. Scheffé confidence intervals were computed for time and treatment means. Values within the time mean row or the treatment mean followed by the same letter do not differ significantly ($p = 0.05$). The 12 wk data analyzed with a one-way ANOVA. Values in the 12 week row followed by the same letter do not differ significantly ($p = 0.05$).

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5. MICROBIAL ACTIVITY, MYCORRHIZAL INFECTION AND PLANT GROWTH RESPONSES TO SEWAGE SLUDGE AMENDATION OF PEAT-TAILING SAND MIXTURES

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5.1. ABSTRACT

Extracted oil sands were mixed with 55% (v/v) peat and amended with dry sewage sludge at rates equivalent to 0, 23, 46 and 92 mT ha^{-1}. Slender wheatgrass and jack pine were planted in the amended oil sands and the plants were grown in the greenhouse for 10 and 15 wk respectively. Growth of both plant species was greatly enhanced by the sewage sludge with maximum growth of jack pine occurring with 23 mT ha^{-1} and maximum growth of slender wheatgrass occurring with 46 mT ha^{-1}. Additional sewage sludge did not affect the growth of slender wheatgrass and a rate of 92 mT ha^{-1} strongly decreased root and shoot growth of jack pine. VA mycorrhizal formation was completely inhibited by all sewage sludge applications while ectomycorrhizal development was severely reduced by sewage. Sewage sludge additions increased basal respiration and microbial biomass in the planting media and decreased the rate of decomposition of slender wheatgrass leaves. The presence of slender wheatgrass increased microbial biomass in the planting media and reduced rates of decomposition. Jack pine had little influence on respiration, microbial biomass or decomposition.

5.2. INTRODUCTION

Sewage sludge can be a highly beneficial amendment on severely disturbed soils or on mine spoils (Berry and Marx, 1977; Visser *et al*., 1984). It has the advantage over mineral fertilizers in that nutrients are slowly released over a period of years thus eliminating the need for successive fertilizer applications. In a previous study, sewage sludge and peat were applied separately to oil sand tailings and both benefited plant growth (Visser *et al*., 1984). In that peat is routinely applied to oil sand tailings but the peat is deficient in P, it was thought that combining peat with its recalcitrant C and sewage sludge with slowly available nutrients might result in a highly desirable growth medium. However, high soil nutrient levels can inhibit mycorrhizal formation and decomposition (Part 3) and sewage sludge does not always have favourable effects on plant establishment (Visser *et al*., 1984). Thus it was of interest to determine the effects of adding different levels of sewage to soil on plant growth and microbial factors.

The objectives of this study were to determine the effects of (1) adding different amounts of dried sewage sludge on plant growth, (2) sewage sludge on decomposition potential, respiration and microbial biomass of a peat-tailing sand mixture, (3) sewage sludge on VA and ectomycorrhizae formation and (4) plants on decomposition potential and microbial biomass.

5.3. MATERIALS AND METHODS

The general procedures used were the same as for the fertilizer experiment (Part 3). A 1:1 (v/v) mixture of Carex and feather moss peats (Part 2) were mixed with a second mixture of tailings sand and silt-clay overburden, with the peat composing 55% (v/v) of the final growing medium. Dry sewage sludge was obtained from the City of Calgary Sewage Treatment plant, broken up and sieved, with the fraction between 2 and 4 mm being retained for use. The sewage sludge was added to the growing medium at a rate equivalent to 46 mT ha^{-1}, the rate used in the tank study (Visser et al., 1984) and at zero, one-half and twice this rate. The sewage sludge was thoroughly mixed with the planting medium and placed in 12.5 cm diameter pots and moistened. The actual amount added in the 46 mT ha^{-1} treatment was 40.5 g per pot. Five replicate pots of each sewage level were planted with three slender wheatgrass seeds per pot, five replicate pots were each planted with three pregerminated jack pine seeds and five replicates were left unplanted. In that it was suspected that the peat from Ft. McMurray contained low levels of ectomycorrhizal inoculum, peat from the Canmore site in the Rocky Mountain foothills (Danielson et al., 1984) was utilized to duplicate the jack pine plant growth and mycorrhizal study. This peat,formed under white spruce, was known to contain high levels of mycorrhizal inoculum. In order to detect airborne inoculum, five replicate, untreated pots of each peat type were autoclaved and planted with jack pine. Pots were watered three times weekly.

When the slender wheatgrass was 10 wk old and the jack pine 15 wk old, the plants were harvested. Tillers of slender wheatgrass were counted and seeds and shoots dried at 80°C and weighed. Roots were subsampled for VA mycorrhizal evaluations and dried and weighed. Jack pine shoots were dried and the roots reserved for mycorrhizal counts.

Slender wheatgrass roots were cleared and stained in 0.01% trypan blue according to Phillips and Hayman (1970) and the degree of infection determined using a line intersect method (Zak and

Parkinson, 1983). Jack pine roots were cut into 2-3 cm segments and 300 short roots per sample were rated for infection (Part 3). To further characterize the mycorrhizae, attempts were made to culture the symbionts.

Basal respiration of the amended planting media was determined by measuring CO_2 efflux under standard conditions of temperature and moisture. An "Ultragas 3" CO_2 analyzer (Wosthoff Company, Bochum, Germany) was used to determine respiration rates. The same samples were used to determine the quantity of microbial biomass C present using the technique of Anderson and Domsch (1978). Decomposition potential was estimated by placing about .25 g of slender wheatgrass leaves in 1 mm mesh litter bags and burying one bag in each pot. When the plants were harvested, the bags were removed, washed and dried to constant weight at 35°C.

5.4. RESULTS

The growth of slender wheatgrass was strongly stimulated by the addition of sewage sludge (Table 1). Growth was very poor in the unamended peat and plants failed to flower. The addition of more than 46 mT ha^{-1} of sewage sludge did not result in any further increases in growth. Shoot/root ratios were very large when grown in the 46 and 92 mT ha^{-1} treatments.

The growth of jack pine was also greatly enhanced when either 23 or 46 mT ha^{-1} rates of application were used (Table 2). The addition of more sewage sludge reduced both shoot and root growth and resulted in very low numbers of short roots. All three levels of application resulted in a small number of short roots per unit root weight. Subordinate mother roots (Wilcox, 1968) were small in number and did not constitute a significant portion of the root systems.

The high level of sewage resulted in a planting medium that was lethal to about 10% of the recently germinated jack pine seedlings. All seedlings survived in pots without sewage. Needles of all the sewage treated seedlings were dark green with no apparent symptoms of nutrient imbalances.

VA mycorrhizal development on slender wheatgrass plants was of a limited extent, with only 9% of the root system being infected after 10 wk (Table 3). Sewage sludge completely inhibited infection by symbionts indigenous to the peat. Root length was substantially reduced by the addition of more than 23 mT ha^{-1} of sewage sludge.

Ectomycorrhizal inoculum compatible with jack pine was present in both peat mixtures resulting in root systems with a large portion of the short roots mycorrhizal (Table 4). However the addition of sewage sludge almost completely prevented the development of mycorrhizae with only 3% of the short roots mycorrhizal in the 23 and 46 mT ha^{-1} treatments. All of the symbionts appeared to be Basidiomycetes except for the I-type Ascomycete. Mycelial strands were formed by the Rhizopogon-type and Amphinema byssoides (Fr.) J. Erikss.

Table 1. Growth of slender wheatgrass in a tailings sand-peat mixture amended with three levels of sewage sludge.

Parameter	Sewage sludge (mT ha^{-1})			
	0	23	46	92
Shoot weight (g)	$.56^a$	5.8^b	7.6^c	7.9^c
Root weight (g)	$.45^a$	2.4^b	1.8^c	1.8^c
Seed weight (g)	0	$.27^a$	$.66^a$	1.6^b
Shoot/root ratio	1.3	2.4	4.2	4.3
Tillers $plant^{-1}$	2.9^a	13.2^b	17.8^c	19.7^c

All data except seed weight ln Y transformed prior to performing one-way ANOVA, difference tested by Scheffé pairwise comparisons. Values in each row followed by the same letter do not differ significantly (p = .05).

Table 2. Growth of jack pine on peat-tailings sand mixtures amended with three rates of sewage sludge.

	Peat source	Sewage sludge (mT ha^{-1})				Autoclaved
		0	23	46	92	
Shoot weight (mg)[1]	Ft. McMurray	121^{a}	811^{c}	1235^{c}	312^{b}	148^{a}
" " "	Canmore	188^{ab}	894^{c}	842^{c}	267^{b}	95^{a}
Root weight (mg)[1]	Ft. McMurray	97^{a}	336^{bc}	583^{c}	163^{ab}	101^{a}
" " "	Canmore	120^{a}	366^{b}	350^{b}	113^{a}	51^{a}
Shoot/Root ratio	Ft. McMurray	1.3	2.4	2.1	2.2	1.5
" " "	Canmore	1.6	2.5	2.5	2.4	1.9
Branches/plant	Ft. McMurray	.5^{a}	2.3^{bc}	2.7^{c}	1.2^{ab}	0
	Canmore	1.2^{a}	2.1^{b}	1.5^{ab}	1.1^{a}	0
Short roots/plant[2]	Ft. McMurray	405^{b}	504^{c}	342^{abc}	104^{a}	477^{c}
	Canmore	378^{abc}	324^{abc}	164^{ab}	125^{ab}	165^{ab}
Short roots/mg	Ft. McMurray	12.3	3.5	1.6	.6	13.6
root[1]	Canmore	9.3	2.5	1.2	.7	9.4
Subordinate mother	Ft. McMurray	2.1	12.4	11.9	3.4	2.3
roots/plant	Canmore	1.5	6.9	11.5	7.4	2.3

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[1] Data analyzed by one-way ANOVA after ln Y transformation and differences tested by Scheffé pairwise comparisons. Values in each row followed by the same letter do not differ significantly ($p = .05$).

[2] Data analyzed by two-way ANOVA and significant differences detected by Scheffé confidence intervals. Values followed by same letter(s) are not significantly different ($p \leq 0.05$).

Table 3. VA mycorrhizal infection and root lengths of slender wheatgrass grown peat amended oil sands with three levels of sewage sludge.

	Sewage sludge (mT ha^{-1})			
	0	23	46	92
Total root length (m l^{-1})	130	840	349	240
Mycorrhizal root length (m l^{-1})				
Total	12.8	0	0	0
With hyphae	7.9	0	0	0
With vesicles	1.5	0	0	0
With arbuscules	3.4	0	0	0
Percent infection ($\bar{x}$ ± SD)	9.2 ± 9.8	0	0	0

Root length data ln Y transformed prior to analysis by one-way ANOVA and differences tested by Scheffé pairwise comparisons. Values followed by the same letter do not differ significantly ($p = .05$).

Table 4. Percent mycorrhizal infection of jack pine seedlings growin
peat-tailing sand mixtures amended with three rates of sewage sludge.

	Peat	Sewage sludge (mT ha^{-1})				Auto-
	Source	0	23	46	92	claved
Total infection	Ft. McMurray	31	0	0	0	0
	Canmore	57	3	3	0	0
I-type Ascomycete	Ft. McMurray	5	0	0	0	0
	Canmore	15	0	3	0	0
Rhizopogon-like	Ft. McMurray	12	0	0	0	0
	Canmore	0	0	0	0	0
Tomentella sp.	Ft. McMurray	0	0	0	0	0
	Canmore	23	3	0	0	0
Amphinema byssoides	Ft. McMurray	0	0	0	0	0
	Canmore	0	0	0	0	0
Hyaline	Ft. McMurray	0	0	0	0	0
Basidiomycete	Canmore	17	0	0	0	0
Unknowns	Ft. McMurray	13	0	0	0	0
Unknowns	Canmore	0	0	.2	0	0

The addition of sewage sludge resulted in increased basal respiration of the planting medium after both 10 and 15 wk (Table 5). Amendation with a rate of 92 mT/ha of sewage sludge resulted in significant increases in CO_2 evolution as compared with a rate of 23 mT ha^{-1} in unplanted pots. The presence of slender wheatgrass did not result in a significant change in respiration at any level of application, but at an application equivalent to 92 mT ha^{-1} respiration was significantly higher in pots planted with jack pine than in unplanted pots. Root weights of jack pine in the 92 mT ha^{-1} treatment were low (Table 2) indicating that factors other than root mass were involved in the stimulation of respiration. The microbial biomass C was increased by sewage amendation and was similar at 10 and 15 wk (Table 6). The amount of microbial biomass in the planting media was greater when planted with slender wheatgrass than in unplanted pots at all three levels of sewage sludge application, but only at the highest level when jack pine was planted. In pots planted with either slender wheatgrass or jack pine, the microbial biomass increased steadily as the rate of sewage sludge application increased although root and shoot biomass did not increase (slender wheatgrass, Table 1) or decrease (jack pine, Table 2).

The decomposition rates of slender wheatgrass leaves were significantly reduced when sewage sludge was added to the growing medium (Table 7). In pots planted with slender wheatgrass, decomposition was strongly reduced and the amount of sewage sludge applied had no significant effects. The presence of jack pine did not affect decomposition rates as much as did slender wheatgrass but with the highest rate of sewage sludge application the weight of leaves remaining after 15 wk was significantly greater in both peat types when jack pine was present than in unplanted pots. The type of peat used to amend the tailings sand had only small effects on the rates of decomposition (Table 8). Autoclaving the peat appeared to decrease the rate of decomposition but not to a substantial extent.

Table 5. Basal respiration of a peat-tailings sand mixture amended with three levels of sewage sludge and planted with slender wheatgrass, jack pine or left unplanted. Values are μl CO_2↑ $100\ g^{-1}\ h^{-1}$ growing medium.

	Sewage sludge (mT ha^{-1})			
Treatment	0	23	46	92
Control (10 wk)	125^{a}	244^{b}	355^{bc}	502^{cd}
Slender wheatgrass	113^{a}	422^{bcd}	568^{cd}	786^{d}
Control (15 wk)	175^{ab}	207^{b}	271^{bc}	398^{c}
Jack pine	121^{a}	191^{ab}	261^{b}	541^{d}

Slender wheatgrass data ln Y transformed. All data analyzed by two-way ANOVA and differences tested by Scheffé pairwise comparisons. Values in each set followed by the same letter do not differ significantly ($p = .05$).

Table 6. Microbial biomass C in a peat-tailings sand mixture amended with three levels of sewage sludge and planted with slender wheatgrass or jack pine or left unplanted. Values are mgC 100 g^{-1} planting medium.

	Sewage sludge (mT ha^{-1})			
Treatment	0	23	46	92
Control (10 wk)	12^{a}	25^{b}	33^{bc}	57^{d}
Slender wheatgrass	12^{a}	46^{cd}	65^{d}	103^{e}
Control (15 wk)	13^{ab}	21^{bc}	28^{cd}	55^{e}
Jack pine	11^{a}	22^{c}	33^{d}	65^{f}

Slender wheatgrass data ln Y transformed. All data analyzed by two-way ANOVA and differences tested by Scheffé pairwise comparisons. Values in each set followed by the same letter do not differ significantly (p = .05).

Table 7. Decomposition of slender wheatgrass leaves buried in a peat-oil sands mixture and amended with three levels of sewage sludge.

	Peat	Sewage sludge (mT ha^{-1})			
Treatment	Source	0	23	46	92
Control (10 wk)	Ft. McMurray	7^{a}	14^{ab}	29^{bc}	29^{bc}
Slender wheatgrass	Ft. McMurray	7^{a}	46^{cd}	51^{cd}	41^{c}
Control (15 wk)	Ft. McMurray	0^{a}	13^{bc}	24^{c}	21^{d}
Jack pine	Ft. McMurray	8^{ab}	15^{bcde}	23^{cdef}	31^{f}
Jack pine	Canmore	14^{bcd}	25^{f}	24^{cdef}	27^{f}

Data analyzed by two-way ANOVA and differences tested by Scheffé pairwise comparisons. Values within each set followed by the same letter do not differ significantly ($p = .05$).

Table 8. Decomposition of slender wheatgrass leaves buried in peat-oil sands mixture, planted with jack pine and amended with three levels of sewage sludge. Values are percent weight remaining.

		Sewage sludge (mT ha^{-1})			
Peat source	Autoclaved	0	23	46	92
Ft. McMurray	22^{bc}	8^{a}	15^{ab}	23^{bc}	31^{c}
Canmore	21^{ab}	14^{a}	25^{b}	24^{b}	27^{b}

Data analyzed by one-way ANOVA and differences tested by Scheffé pairwise comparisons. Values in each row followed by the same letter do not differ significantly (p = .05).

5.5. DISCUSSION

Sewage sludge served as an excellent source of plant nutrients for both slender wheatgrass and jack pine in this greenhouse pot study. However heavy applications resulted in either no benefit for growth or, in the case of jack pine, severely decreased primary production and survival of young seedlings. In addition, even the lowest level of application used resulted in a nearly complete suppression of mycorrhizal formation. The absence of mycorrhizae and their extensive network of extramatrical mycelium in conjunction with the reduction of root growth with high sewage sludge levels could result in severe consequences for plant establishment on minespoils where a variety of stresses, in addition to macronutrient deficiencies, may occur. In this study neither shoot nor root growth of slender wheatgrass gave any clear indication that the amount of sewage sludge might have any impact on tolerance to non-nutrient stresses. However a measure of root length, as well as mycorrhizal development, provides evidence that grasses subjected to high nutritional levels are probably less able to exploit soil or mine spoils for either nutrients of low mobility or water. Unlike an uncontrolled reclamation situation, these stresses are unlikely and probably underestimated within pots in greenhouse conditions.

Much heavier field applications of sewage sludge have been used without the negative effects described here. Berry and Marx (1976) used up to 275 mT ha^{-1} and did not find any adverse effects on either plant growth or mycorrhizal infection of two species of southern pines. These authors recommended that application rates on disturbed sites should be as high as environmental and economic considerations permit. In a later study, Berry and Marx (1977) found that growth of loblolly pine and mycorrhiza formation were depressed by adding 275 mT ha^{-1} of sewage sludge. However growth was excellent with rates similar to those used in this study. However, rates applied to pots and to field plots are not necessarily comparable. The sewage sludge used in the pots was in the form of small pellets, thoroughly mixed into the planting mixture and was kept constantly wet by frequent watering.

In contrast, field applied sludge may be in large chunks and some portion is on the surface where it is dry for much of the growing period. Regardless, the present pot study illustrated that high application rates can have highly deleterious effects on root growth and mycorrhizae. When evaluating the effects of sewage sludge, root length rather than weight, should be measured as well as root associated symbioses.

As expected, sewage sludge additions increased respiration and microbial biomass. It is presumed that the increase in respiration and microbial biomass was due to decomposition of material in the sewage sludge rather than a stimulation of peat decomposition. The addition of mineral nutrients to peat has been shown to have no effect on these parameters (Part 2). The presence of slender wheatgrass doubled the amount of microbial biomass in the sewage treated planting medium. This indicates that a large portion of the microbial biomass was more active in the decay of easily degraded substances (sloughed roots, root exudates) rather than in the decay of peat or recalcitrant materials in the sewage sludge. It is possible that, as was indicated by the inhibition of litter decomposition, sewage sludge may even inhibit decay of recalcitrant materials.

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6. PLANT GROWTH, MICROBIAL ACTIVITY AND CELLULOSE DECAY IN TAILING SAND TREATED WITH THREE LEVELS OF SEWAGE SLUDGE

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6.1. ABSTRACT

In a greenhouse pot experiment, cxtracted oil sand was left unamended or amended with dried sewage sludge at levels equivalent to 23, 46 and 92 mT ha^{-1} and subsequently planted with slender wheatgrass or left unplanted. Pots were sampled at 2, 6 and 12 wk to determine effects of sewage sludge on plant growth, microbial activity, mycorrhizal infection and cellulose decay potential. Shoot and root production were most enhanced by the 23 and 46 mT ha^{-1} sludge rates and were significantly reduced by the 92 mT ha^{-1} application. Over the first 6 wk the 46 mT treatment also had adverse effects on plant growth, but this inhibition was overcome during the following 6 wk. No vesicular-arbuscular (VA) mycorrhizal infection was detected in any of the treatments. Microbial respiration and biomass C increased with increasing sludge levels and were not substantially affected by the presence of plants. The addition of sewage sludge stimulated cellulose decay potential which was observed to be negligible in the untreated sand.

6.2. INTRODUCTION

Sewage sludge has been shown to be highly effective in ameliorating soil chemical/physical properties and promoting plant establishment and growth in nutrient-impoverished minespoils (Sopper, 1970; Berry and Marx, 1977; Stout et al., 1978; Bradshaw et al., 1978; Visser et al., 1984a). Application of sewage sludge to oil sand tailings, which are characterized by an absence of organic matter, essential plant nutrients and a microbial component, will improve both the nutrient and microbial status of the sand (Visser et al., 1984b). Also, because of its slow decay rate, sewage sludge behaves as a slow release fertilizer, making it a highly desirable amendment for reclaimed minespoils requiring a minimum of fertilizer maintenance. However, high levels of sewage may have adverse effects on plant establishment and growth by inhibiting seed germination and root elongation (Wong et al., 1981; Sabey and Hart, 1975; Wollen et al., 1978; McCormick and Borden, 1973). VA mycorrhizal infection rates also appear to be adversely affected when plants are grown in the presence of sewage sludge (Spitko and Manning, 1981; Zak and Parkinson, 1982), while high soil fertility levels can reduce soil microbial activity (Kowalenko et al., 1978). It was, therefore, believed necessary that additional information be obtained on the effects of different sewage sludge levels on plant response and microbiological parameters in tailings sand. The main objectives of this study were to determine (1) the effects of different levels of sewage sludge applied to tailing sand on primary production of slender wheatgrass, (2) the effect of sewage sludge on microbial respiration and biomass C, (3) the effect of sewage sludge on the decay of cellulose filter paper, (4) the effects of plant growth on soil microbial activity and cellulose decomposition, and (5) the VA mycorrhizal infection potential of sludge treated sand.

6.3. MATERIALS AND METHODS

Anaerobically digested sewage sludge from the City of Calgary sewage lagoons was air dried and sieved to produce pellets 2 to 4 mm in diameter. The dried sludge had a pH of 8.6 and contained approximately 30% organic C, 3% N (of which .32% was NH_4-N), 15,325 $\mu g\ g^{-1}$ P (of which 170 $\mu g\ g^{-1}$ were extractable), 11,950 $\mu g\ g^{-1}$ Fe, 815 $\mu g\ g^{-1}$ Cu, 1525 $\mu g\ g^{-1}$ Zn and 788 $\mu g\ g^{-1}$ Pb.

The sewage sludge pellets were thoroughly mixed with tailings sand (pH 7.5; .15% organic C, < .01% N, 1.1 $\mu g\ g^{-1}$ extractable P, 2.9 $\mu g\ g^{-1}$ Fe, .05 $\mu g\ g^{-1}$ Cu, 0.3 $\mu g\ g^{-1}$ Zn, and .5 $\mu g\ g^{-1}$ Pb) to obtain treatments equivalent to the following application rates: 0 (no sewage sludge); 23 mT ha^{-1}; 46 mT ha^{-1}; 92 mT ha^{-1}. The 46 mT ha^{-1} rate was equivalent to the rate applied to the tailing sand in the tank study (see Visser et al., 1982a). To avoid variation due to subsampling, each treatment was set up on a per pot basis so that the 23 mT treatment contained 2.66% sludge/pot (w/w); the 46 mT treatment contained 5.32% sludge/pot and 92 mT treatment contained 10.64% sludge/pot. In addition to improving the nutrient quality of the sand, the sludge also raised the moisture holding capacity of the sand from 20% in the untreated sand to 22%, 24% and 26% in the 23, 46 and 92 mT treatments respectively. The mixtures for each pot were remoistened with distilled water to 75% of field capacity. Nine replicate pots of each sewage sludge treatment were planted with five, 1 wk old slender wheatgrass seedlings and six replicates $treatment^{-1}$ were left unplanted. Plant growth parameters were measured at 2, 6 and 12 wk after planting and microbial respiration, microbial biomass C, and cellulose decay potential were assessed at 6 and 12 wk after planting. Three planted and then unplanted pots $treatment^{-1}$ were destructively sampled at each sampling time with the exception of the 2 wk samples when only planted pots were processed. Pots were incubated in the greenhouse and maintained at 75% field capacity with distilled H_2O during the experiment.

At each sampling time, the number of tillers produced by each grass plant was determined and shoots were dried at 80°C and weighed. Roots were washed free of planting mixture, dried and weighed. On the last sampling date, roots were subsampled, cleared, and stained in 0.01% trypan blue (Phillips and Hayman, 1970) for vesicular-arbuscular mycorrhizal evaluations.

Sand from the planted and unplanted pots, harvested 6 and 12 wk after planting, was analyzed for microbial activity and microbial biomass C. Microbial activity (CO_2 efflux) was determined under standard temperature and moisture conditions using an "Ultragas 3" CO_2 analyzer (Wösthoff Co., Bochum, Germany). The same samples were used to determine the quantity of microbial biomass C using the technique described by Anderson and Domsch (1978). Details for determining soil respiration and microbial biomass C have been outlined by Visser et al. (1982b). The cellulose decay potential of each planted and unplanted sand/sludge mixture was estimated at 6 and 12 wk after planting by bagging a pre-weighed 4.5 cm diameter filter paper in 1 mm nylon mesh and embedding one filter in each pot. At harvest, the bags were removed, washed, dried at 35°C and percent dry weight remaining for each filter calculated.

6.4. RESULTS

The poor nutrient status of the tailings sand resulted in very little shoot and root production by slender wheatgrass over the term of the study (Table 1). At 2 wk, plants in the untreated tailing sand appeared healthy with no obvious nutrient deficiencies but as growth progressed seedlings exhibited stunting and possible N and P deficiencies.

Plant production was most stimulated by the application of sewage sludge at the 23 and 46 mT ha^{-1} rates and significantly depressed by the 92 mT ha^{-1} application (Table 1). Six weeks after planting, shoot and root weights demonstrated a decreasing trend with increasing sludge levels, but at 12 wk this trend was not as pronounced. Although slender wheatgrass exhibited very little growth in the 92 mT treatment over the first 6 wk, growth was significantly stimulated between 6 and 12 wk. Six week-old plants in the high sludge treatments were stunted with yellowish foliage, but at 12 wk plants appeared healthier and exhibited stimulated growth. Shoot/root ratios measured at all three sampling times demonstrated an increasing trend with increasing sludge levels. Tiller production occurred between 6 and 12 wk after planting and was most enhanced by the 23 and 46 mT ha^{-1} application rates. Seedling mortality was greatest in the two high sludge treatments where 8% of the total number of seedlings died over the term of this study (2% were lost in the control treatment and none in the 23 mT ha^{-1} treatment).

Vesicular-arbuscular mycorrhizal infection was not detected in 12 wk old roots from any of the treatments.

Microbial respiratory activity demonstrated an increasing trend with increasing sludge application rates, while microbial biomass C increased significantly with each successive sludge level (Tables 2 and 3). The presence of slender wheatgrass at either 6 or 12 wk after planting had no significant influence on soil respiration, but resulted in a small but significant increase in microbial biomass C when results from both sampling times were

Table 1. Growth of slender wheatgrass in tailings sand amended with three levels of sewage sludge.

Plant parameter	Plant age (weeks)	Sludge application (mT ha^{-1}) 0	23	46	92
Shoot wt (mg plant^{-1})	2	3.3^{ab}	3.2^{ab}	2.3^{ab}	1.5^{a}
	6	6.4^{bc}	52.2^{ef}	20.5^{de}	4.1^{abc}
	12	9.6^{cd}	240.1^{gh}	357.2^{h}	82.3^{fg}
Root wt (mg plant^{-1})	2	3.4^{bc}	0.9^{ab}	0.4^{a}	0.2^{a}
	6	8.1^{cd}	17.8^{de}	6.6^{cd}	0.8^{ab}
	12	5.7^{cd}	72.5^{ef}	82.8^{f}	14.2^{cd}
Shoot/root ratio	2	1.0	3.6	5.8	7.5
	6	0.8	2.9	3.1	5.1
	12	1.7	3.3	4.3	5.8
Tillers plant^{-1}	2	1^{a}	3.2^{ab}	2.0^{ab}	1^{a}
	6	1^{a}	8.5^{c}	10.3^{c}	4.3^{b}

Data in each of the shoot wt., root wt. and tiller categories were analyzed by a two-way ANOVA and Scheffé multiple contrasts for pairwise comparisons. Values followed by the same letter(s) do not differ significantly ($p < 0.001$). Shoot and root wt. data required ln Y transformation.

Table 2. Microbial respiration (μl CO_2 † 100 g^{-1} dry soil h^{-1}) of tailing sand treated with three levels of sewage sludge and left unplanted or planted with slender wheatgrass.

Sludge level ($mT\ ha^{-1}$)	Sampling time			
	6 weeks		12 weeks	
	unplanted	planted	unplanted	planted
0	49^{a}	57^{ab}	57^{ab}	57^{ab}
23	180^{cde}	172^{cde}	139^{abc}	155^{bcd}
46	270^{ef}	351^{fg}	270^{ef}	245^{de}
92	351^{fg}	425^{g}	400^{g}	425^{g}

Data analyzed by three way ANOVA (MSE = 500). Differences detected by Scheffé multiple contrasts for pairwise comparisons. Values followed by same letter(s) do not differ significantly ($p \leq 0.05$).

Table 3. Microbial biomass C (mg 100 g^{-1} dry soil) in tailing sand treated with three levels of sewage sludge and left unplanted or planted with slender wheatgrass.

Sludge level ($mT\ ha^{-1}$)	Sampling Time					
	6 weeks			12 weeks		
	Unplanted	Planted	$\bar{x}$	Unplanted	Planted	$\bar{x}$
0	5.3	4.3	4.8^a	4.3	4.6	4.5^a
23	15.8	21.6	18.7^c	11.8	13.8	12.8^b
46	24.3	28.8	26.6^d	20.0	22.6	21.3^c
92	32.8	32.4	32.6^e	30.8	35.0	32.9^e

	Combined 6 and 12 week unplanted $\bar{x}$	Combined 6 and 12 week planted $\bar{x}$
All treatments	18.1^a	20.4^b

Data analyzed by three way ANOVA (MSE = 5.2). Planted and unplanted means at each sampling time were combined to determine sludge level and time effects, while all means in the unplanted or planted treatments were combined to test plant effects. Differences were detected using Scheffé multiple contrasts for pairwise comparisons. Values in the mean columns in each data set, followed by same letter do not differ significantly ($p \leq 0.05$).

combined. With the exception of the 46 mT ha^{-1}, planted treatment where CO_2 efflux decreased substantially between the 6 and 12 wk sampling times, no changes in microbial respiration were detected over the course of the study. However, microbial biomass C in the 23 and 46 mT ha^{-1} planted and unplanted treatments decreased between 6 and 12 wk after the initiation of the study. Microbial biomass C in the control and 92 mT ha^{-1} sludge treatments were not significantly altered over time.

No decomposition of cellulose filter paper occurred in the untreated tailings sand over the 12 wk study period (Table 4). In general, sewage sludge application stimulated the decay of the filters. The presence of plants did not influence the cellulose decay rate at any of the sludge levels, and amount of filter paper remaining after 6 wk decay was not significantly different from that remaining after 12 wk decomposition. The decomposition data were highly variable - a factor which may have been due to the heterogeneous distribution of sludge pellets within the sand matrix.

Table 4. Decay of cellulose filter paper in tailings sand amended with three levels of sewage sludge and left unplanted or planted with slender wheatgrass.

Sludge level (mT ha^{-1})	% dry weight filter paper remaining			
	6 weeks		12 weeks	
	Unplanted	Planted	Unplanted	Planted
0	100^{c}	100^{c}	100^{c}	100^{c}
23	50^{abc}	18^{ab}	38^{ab}	3^{a}
46	16^{ab}	30^{ab}	38^{ab}	20^{ab}
92	37^{ab}	56^{bc}	63^{bc}	39^{ab}

Data analyzed by three-way ANOVA (MSE = 140). Differences detected by Scheffé multiple contrasts for pairwise comparisons. Values followed by same letter(s) do not differ significantly ($p \leq 0.05$).

6.5. DISCUSSION

The infertility of the tailing sand which, according to Turchenek (1976) is mainly due to a lack of organic matter and inorganic colloids, resulted in a very poor performance by slender wheatgrass. Similar results were obtained when growing slender wheatgrass in spent tailing sand held in large wooden tanks in the field (Visser et al., 1984a). The addition of sewage sludge to the extracted sand at rates equivalent to 23, 46 and 92 mT ha^{-1} increased the organic matter (as determined by loss on ignition) level of the sand to 1.2, 1.5 and 2.1%, respectively. The increase in organic matter levels would be instrumental in improving the nutrient status and water retention capability of the sand. Amendation of the spent sand with the nutrient rich sewage sludge was highly effective in stimulating plant growth, but only at the 23 and 46 mT ha^{-1} application rates. The addition of 92 mT ha^{-1} sewage sludge severely reduced the growth of the slender wheatgrass, and there was some indication that over the first 6 wk of the study the 46 mT application may also have inhibited plant growth. Toxic effects of sewage sludge on plant growth have been reported in other studies (Wong et al., 1981; Sabey and Hart, 1975; Wollen et al., 1978 and McCormick and Borden, 1973), and have been attributed to the presence of toxic concentrations of ammonia after sludge application (McCormick and Borden, 1973). The improvement in plant growth between 6 and 12 wk in the 92 mT ha^{-1} sludge treatment may be explained by the increased microbial oxidation of the ammonia to nitrate, thereby reducing plant inhibitory levels of ammonia. Interestingly, when the same levels of sewage sludge used in this study, were applied to a 55% (v/v) peat/sand mix and planted with slender wheatgrass, plant growth was not significantly reduced by the 92 mT ha^{-1} sludge treatment (see Part 5, this report). The high adsorptive capacity, chelating properties, and nitrification capability of peat may have lessened the inhibitory effects of the higher sludge levels on grass growth in the peat/sand mix. The increased supply of N provided by the sewage sludge was evidenced in the of the size of the grass root systems and increase in shoot/root ratios, as sludge levels increased (Russell, 1977). As suggested in Part 5 the very small root systems produced by grasses subjected to high fertility regimes, may not be advantageous in a minespoil such as the tailing sand, where a well-developed fibrous root system would provide both better erosion control and retention of key nutrients within the soil/plant system.

The lack of VA mycorrhizal infection of the grass roots was to be expected since Zak and Parkinson (1982) have observed that oil sands tailings are essentially devoid of VA mycorrhizal inoculum. It is difficult to determine if the sewage sludge contained any mycorrhizal inoculum, since there is some evidence that sludge may be highly toxic to the VA mycorrhizal fungi (Spitko and Manning, 1981; Zak and Parkinson, 1982).

Microbial respiration and biomass C were low in the spent sand mainly because the spent sand lacks the organic matter required by the microorganisms for their growth and maintenance. The addition of sewage sludge not only improves the organic C and nutrient status of the sand, but also introduces large quantities of bacteria and fungal hyphae (Visser et al., 1984b). Hence it is not surprising that in this study, microbial respiration and biomass C rose considerably with the application of the sludge and increased as sludge levels increased. Macgregor and Naylor (1982) and Eiland (1981) have also noted that microbial respiration and biomass were higher in soil receiving sewage sludge applications. The presence of slender wheatgrass in the sludge-treated sand resulted in a stimulation of microbial biomass, but not to the same extent as that observed in the sludge-treated peat/sand mixture (Part 5). It has been postulated that much of the stimulation of microbial activity and biomass often observed in planted versus unplanted systems can be attributed to the input of easily degradable resources in the form of sloughed roots and root exudates (Visser et al., 1984b). Slender wheatgrass grown in the sludge amended peat/sand mixture produced over four times as much root material as slender wheatgrass grown in the sludge-treated tailing sand. The greater root mass produced in the peat/sand presumably resulted in a larger input of root exudates which would account for the greater stimulation of soil microbial biomass in this treatment.

Untreated tailings sand exhibited no cellulose decay potential presumably because both the nutrients and microorganisms required for the degradation of pure cellulose were absent. The addition of sewage sludge with its high inoculum level greatly accelerated cellulose decay. Cellulolytic bacteria have been isolated from sewage sludge (Gude, 1980; Ramasamy et al., 1981), hence their presence in the sludge used in this study may explain the faster decomposition of cellulose in the sludge treated than untreated sand.

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7. SUMMARY

From the data collected in the greenhouse pot studies, it is possible to make the following conclusions.

7.1. PLANT GROWTH AND MYCORRHIZAL STATUS

Although differences in plant growth in the feather moss peat, *Carex* peat and mixture of these two peats were minimal, there was some suggestion that slender wheatgrass root production and jack pine root and shoot production were superior in 55% (v/v) peat/mineral mixtures. The 55% peat/sand mixture has a field equivalent of 38 mT feather moss peat ha^{-1}, 48 mT *Carex* peat ha^{-1} or 45 mT peat mixture ha^{-1}. The application of mineral fertilizer at the initiation of the study may have obscured any growth differences caused by the different peat types.

Fertilization of the peat, up to a total of 112:49:72 kg NPK ha^{-1}, substantially increased both shoot and root growth by slender wheatgrass. Shoot growth by jack pine reached a maximum at 28:12:18 kg NPK ha^{-1} and was not further stimulated by additional fertilization. Both jack pine root growth and ectomycorrhizal infection of these roots were reduced at fertilizer levels above the 28:12:18 kg NPK ha^{-1} rate. There were negligible quantities of VA inoculum in the peat/sand mixture as evidenced by the lack of mycorrhizal infection of the slender wheatgrass roots in the unfertilized and low fertilizer treatments.

Successful establishment, growth, and seed production by alsike clover in the feather moss peat used in the field study (Visser *et al*., 1984) required the application of additional P. The low extractable P levels in this peat (2 $\mu g\ g^{-1}$) did not appear to be adequate to ensure the establishment of this species during the early stages of plant growth.

Growth of slender wheatgrass and jack pine in a 55% (v/v) peat/tailing sand mixture was greatly stimulated by the addition of dried sewage sludge with maximum shoot production occurring at 46 and 23 m^{T} ha^{-1} sludge levels for grass and pine respectively. The growth of jack pine was strongly inhibited at 92 mT sludge ha^{-1}, while root production by slender wheatgrass decreased sharply at sludge application rates exceeding 23 mT ha^{-1}. When slender wheatgrass was grown in pure tailings sand amended with dried sewage sludge, maximum growth occurred with the 23 mT ha^{-1} sludge rate and was significantly reduced with a sludge application of 92 mT ha^{-1}. These data suggest that the degree of inhibition by sewage sludge on plant growth is dependent not only on the particular plant species but also on the chemical/physical properties of the soil to which the sludge is applied. The adsorptive and chelating properties of peat appeared to decrease the inhibitory properties of sewage sludge. The presence of sewage sludge severely reduced ectomycorrhizal infection of pine roots and completely inhibited VA mycorrhizal infection of the grass roots. It is suggested that the inhibitory effects of sewage sludge may be a result of the generally high nutrient levels characteristic of sludge, the presence of heavy metals in the sludge, or high ammonium N levels which may be particularly problematical immediately after sludge application. Additional research is required to more accurately assess which characteristics of the sludge are most detrimental to plant growth and mycorrhizal formation.

7.2. MICROBIAL ACTIVITY AND DECOMPOSITION

Soil microbial activity was not significantly different amongst the feather moss, Carex and mixed peats, suggesting that the two peat types were very similar in terms of their stability. However, microbial biomass C and decay of grass roots were lowest in the Carex peat. Microbial respiration and biomass were significantly stimulated by the growth of slender wheatgrass, while decomposition potential was reduced in the presence of the grass. These data suggest that mineralization of dead plant parts would be slowest in Carex peat planted with slender wheatgrass.

Amendation of a 55% (v/v) peat/sand mixture with mineral fertilizer up to a total of 112:49:72 kg NPK ha^{-1} did not accelerate the loss of stable C from the peat. Decomposition of slender wheatgrass leaves was reduced by high levels of fertilizer. Microbial biomass was significantly stimulated by the growth of slender wheatgrass in peat with all but the highest fertilizer application (i.e. 112:49:72 kg NPK ha^{-1}). Again, the presence of slender wheatgrass reduced the decay of slender wheatgrass litter.

When P alone was added (rates = 50 and 100 kg P ha^{-1}) to a feather moss peat used in a previous field study (Visser et al., 1984), respiratory activity and microbial biomass were significantly reduced, while decay of pure cellulose was enhanced.

Application of sewage sludge to pure tailings sand or a 55% (v/v) peat/sand mixture at rates ranging from 23 to 92 mT ha^{-1} was highly effective in increasing both microbial respiration and biomass C. This increase is believed to be due mainly to the introduction of high levels of microbial material with the sludge. The growth of slender wheatgrass in the sludge treated peat stimulated the microbial biomass to an even greater degree, but both high levels of sewage sludge and the presence of slender wheatgrass reduced the decomposition rate of slender wheatgrass leaves. It is interesting to note that in each of three separate experiments, the growth of slender wheatgrass inhibited the decay of slender wheatgrass litter - a factor which may be important in newly-revegetated minespoils where a high rate of nutrient turnover may be required to maintain the established vegetative cover. Also, very high nutrient concentrations in either inorganic or organic form may have adverse effects on nutrient release from decaying litter.

It is difficult to apply results obtained under controlled laboratory conditions to a field situation, but the data obtained in the present studies suggest that:

1. with a high initial application of fertilizer the type of peat or amount of peat has very little influence on tree or grass growth over the short term.

2. heavy application of mineral or organic fertilizers may have adverse effects on plant growth, with grasses being less

sensitive to high nutrient regimes than pine. In general, root growth was reduced when high fertilizer rates were used. This may be a disadvantage during the initial stages of revegetation when poor root development could lead to a greater degree of erosion on unstable slopes. Also, plants established in disturbed sites where chemical/physical stresses are high may require extensive root systems in order to survive such adverse conditions as high temperatures, low moisture or low nutrient availability.

3. it appears that many of the peats found in the Fort McMurray area are severly lacking in VA mycorrhizal inoculum. Both VA- and ectomycorrhizal infection of plant roots are inhibited by high nutrient regimes.

4. the decomposition rates of dead plant material may also be reduced by high soil nutrient levels.

5. although growth by slender wheatgrass stimulated microbial respiration and biomass C, decomposition rates were inhibited by the presence of slender wheatgrass. This suggests that during the early stages of reclamation nutrient release from decaying plant material may be more rapid in areas vegetated with trees than in those planted with grass.

7.3. LITERATURE CITED

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ABSTRACT

The vesicular-arbuscular mycorrhizal (VAM) development of slender wheatgrass on extracted oil sands tailings and a subalpine coal mine spoil amended with either fertilizer, peat, or sewage sludge was examined over a 4 yr period. During the first growing season on the oil sands spoil mycorrhizae were limited to plants on the peat-amended spoil. While VAM infection was not detected in plants on the fertilized plots until the end of the second growing season, plants on the sewage- amended plots were not mycorrhizal until after the 4th yr. VAM infection in plants on the subalpine mine spoil was detected at 2 wk only in the peat-amended spoil. Although sewage initially suppressed the rate of mycorrhizal development, plants did develop VA mycorrhizae by the 10 wk sampling time. The mycorrhizal status of plants on the amended subalpine mine spoil did not change significantly between the 2nd and 4th year.

Glomus aggregatum and *Glomus mosseae* were the most common VA fungi in the amended spoils. In the oil sands tailings, VA fungal spores were detected only in the control and peat-amended plots. While there was no amendment effect on spore densities of *G. mosseae* in the subalpine coal spoil, spore numbers of *G. aggregatum* were significantly reduced in the sewage-treated spoil. The successful reestablishment of VA mycorrhizal in mine spoils will depend in part on the effects of soil amendments on VA fungal species occurrence and inoculum production.

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1. INTRODUCTION

The restoration of biological activity in soils disturbed through mining activity may involve the addition of nutrients, some form of organic matter, and the reintroduction of specific groups of decomposer and symbiotic microorganisms (Parkinson 1979). The type of amendment applied to a spoil and its rate of application may influence the development of the saprophytic microflora and the success in reestablishing the necessary symbiotic component. Eiland (1981) showed that the addition of slurry and farmyard manure to an agricultural soil every 2 yr resulted in higher fungal numbers than when applied every year. Within the context of mine spoil reclamation, very little information is available concerning the effects of nutrient addition or organic matter input on the soil microflora of these disturbed habitats.

It is now well recognized that the symbiotic associations of a specific group of phycomycetous fungi with higher plants, termed vesicular-arbuscular mycorrhizae (VAM), are ubiquitous in nature (Mosse 1973a; Crush 1975; Read *et al.* 1976; Davidson and Christenson 1977; Molina *et al.* 1979; Sparling and Tinker 1978a; Rabatin 1979) and that this symbiosis is required by many plants for absorption of phosphorus and other nutrients from soils of low nutrient status (Mosse 1973b; Crush 1976; Powell 1977a; Sparling and Tinker 1978b; Nicolson and Johnston 1979). Recent work by Allen *et al.* (1981) with blue gramagrass has shown that VA mycorrhizal plants may also be more drought tolerant; an important characteristic to consider in the reclamation of semi-arid environments. The occurrence of VAM associations with plants grown on mine spoils has been documented in studies by Daft and Nicolson (1974), Daft and Hacskaylo (1976), Khan (1978), Miller (1979), Reeves *et al.* (1979) and Allen and Allen (1980). Growth increases of mycorrhizal plants as compared with nonmycorrhizal ones on mine spoils have been demonstrated for many plant species (e.g. Aldon 1975; Daft and Hacskaylo 1977; Lindsey *et al.* 1977; Lambert and Cole 1980; Khan 1981).

Given that VA mycorrhizae are an integral component of most terrestrial ecosystems, if reclamation of disturbed habitats is to be effective, attention should be given to the rates at which plants become infected with VA fungi in these habitats and the means of enhancing the mycorrhizal status of plants used for the revegetation of mine spoils. The reestablishment of mycorrhizal relationships has come to be recognized as an important component of an overall reclamation strategy (e.g. Marx 1975; Aldon 1978; Reeves *et al*. 1979; Trappe 1981). The successful development of mycorrhizae in disturbed soils may be influenced by the type of inorganic and organic amendment applied and the frequency of application. Studies on the addition of N or P fertilizer on VA mycorrhizal development in agricultural systems (e.g. Hayman *et al*. 1975; Kruckelman 1975) have shown a decrease in VA infection following application. However, Sparling and Tinker (1978) found that the addition of a N fertilizer to a grassland soil (125 kg · ha) did not alter the amount of mycorrhizal infection. The effects of fertilizer application to mine spoils on VA mycorrhizae have received no attention, but it is presumed that the effects of fertilizer application on the development of VA mycorrhizae depend largely on nutrient levels in the spoil prior to fertilization (Hayman 1978). While numerous studies have examined the effects of sewage sludge on plant growth (e.g. Gaynor and Halstead 1976), there is only meager information concerning mycorrhizal development on sewage amended soils. Berry and Marx (1976) found that sludge application rates as high as 275t dry wt · ha did not reduce the ectomycorrhizal development of *Pinus echinata* Mill. and *P. taeda* L. on severly eroded soils by *Pisolithus tinctorius* (Pers.) Coker and Couch. However, Spitko and Manning (1981) reported that a single application of sewage sludge (4.7 and 9.4t dry wt · ha) on agricultural soils inhibited VA mycorrhizal development of onion.

The potential use of various inorganic and organic surface amendments in mine spoil reclamation warrants a critical examination of their effects, over the long term, on the rates of mycorrhizal development and the status of the infection of plants used for this reclamation. Time-course studies of VA mycorrhizal development have dealt mainly with agronomic plant species growing under either controlled environmental conditions (Beveage and Bowen 1975; Sanders et al. 1977) or in agricultural habitats (Sutton 1973; Rich and Bird 1974; Saif 1977; Black and Tinker 1979). Apart from the investigation by Ponder (1979) which examined the rate of mycorrhizal development of three plant species grown in recently graded mine spoil under greenhouse conditions, there has only been the study by Allen and Allen (1980) which has followed the mycorrhizal development of herbaceous plants grown on mine spoils under field conditions. They found that infection levels in plants grown on topsoil amended mine spoil in Wyoming increased gradually over a 3 yr period following reclamation to within 50% of the infection levels in plants from undisturbed sites.

The rates at which plants develop VA mycorrhizae in habitats disturbed through mining activity will depend in part on the inoculum density and species composition of the VAM fungi in the mine spoil following reclamation. The VAM inoculum density and species composition of these disturbed habitats will be governed by reclamation practices (Allen and Allen 1980; Rives et al. 1980), plant species composition of the reclaimed site (Miller 1979; Reeves et al. 1979), chemical and physical characteristics of the spoil, and the rate of recolonization by mycorrhizal fungi from undisturbed areas around the mine.

The objectives of this study were:

1. To examine the vesicular-arbuscular mycorrhizal development of slender wheatgrass grown on extracted oil sands tailings and a subalpine coal mine spoil over a 4 yr period.
2. To determine the effects of amendation of these mine spoils on the rates of mycorrhizal development and the status of the infection over time.
3. To examine the effects of amendation on the occurrence VAM fungal species and inoculum densities.

This investigation was one aspect of a larger project which examined the effects of amendation, from an organismic and process perspective, on the reinstatement of biological activity in soils disturbed through mining activity (see Danielson _et al._ in prep. and Visser _et al._ in prep. for information pertaining to other aspects of this project).

2. MATERIAL AND METHODS

2.1 EXPERIMENTAL DESIGN OF SPOIL TANK STUDY

The mine spoils chosen for this investigation were the extracted oil sands from the Great Canadian Oil Sands Ltd. processing plant located near Fort McMurray, Alberta and from a pit coal mine located in a subalpine spruce-fir habitat at Luscar, Alberta. Large quantities of each spoil were transported to a location near the University of Calgary in early 1977 and placed in wooden tanks which were subdivided in 16 subunits (5x7x1m) by wooden partitions (Figure 1). Only 12 subunits were used in this investigation. The soil tanks were built into the ground and had an open bottom in the gravel subsoil beneath the original topsoil layer at the site. Approximately 60 cm of spoil was placed in each subunit. After leveling, each subunit was randomly amended only once with either: 1) fertilizer (a mixture of 23-23-0 and 0-0-62) applied at a rate of 113 kg N, 113 kg P_2O_5 and 91 kg $K_2O \cdot$ ha); 2) a feather moss peat from a mountain site near Canmore, Alberta currently dominated by white spruce (applied to a uniform depth of 14 cm). The peat was removed from the site by a front-end loader, after the surface layer was stripped off to avoid introducing weeds, and transported to Calgary by truck. The material was stockpiled for one wk and then applied to the appropriate subunits by hand; 3) sewage sludge (anaerobically digested, applied at a level equivalent to 46 t (dry) · ha, or 4) left unamended. Three subunits were established per treatment. These amendments and application rates were chosen based on either literature information or current reclamation procedures at the mine sites. All amendments were rototilled into the spoils to a depth of 15 cm prior to planting and sampling for chemical analysis.

Immediately following application of the amendments in June 1977 each subunit was divided into four plots (2.75 x 1.75 m), with 0.5 m walkways between them and the walls of the tank, and planted with four plant species:

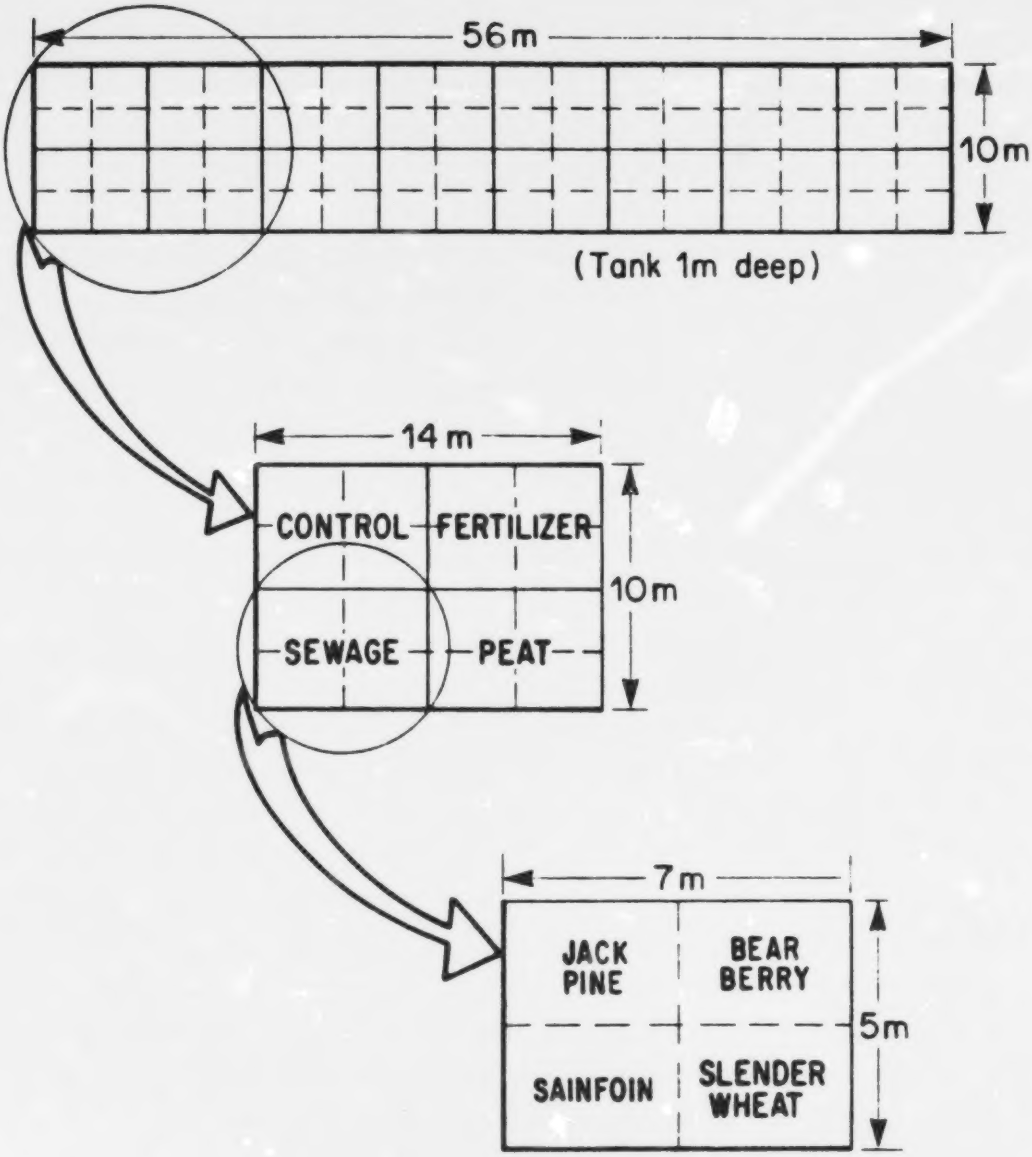
56m
10m
(Tank 1m deep)
14 m
CONTROL
FERTILIZER
10m
SEWAGE
PEAT
7m
JACK PINE
BEAR BERRY
5m
SAINFOIN
SLENDER WHEAT

Figure 1. Plan of spoil tank.

Spoil Type	Plant Species
Oil Sands	*Arctostophylos uva-ursi* (L.) Spreng. (bearberry)
	Pinus banksiana Lamb (jack pine)
	Agropyron trachycaulum (Link) Malte. (slender wheatgrass)
	Onobrychis corniculatus L. (sainfoin)
Subalpine	*Picea glauca* (Moench.) Vass (white spruce)
	Salix sp. (willow)
	Agropyron trachycaulum
	Trifolium hybridum L. (alsike clover)

The plant species chosen for each spoil type represented species that were either being used in revegetation programs at the mine sites and at other locations in the province or were species that occurred in the undisturbed areas around the mines. Slender wheatgrass was chosen for this investigation since it was grown on both mine spoils.

Bearberry, jack pine, white spruce, and willow were planted out as seedlings. Known weights of seed of alsike clover, sainfoin, and slender wheatgrass were hand sown in each plot (Table 1). Plots were weeded and pathways kept clear of vegetation during each growing season.

2.2 SAMPLING PROGRAM AND QUANTIFICATION OF VESICULAR-ARBUSCULAR MYCORRHIZAE

To assess the initial rate of VAM development in roots of slender wheatgrass, five intact plants were dug from each plot (three plots per treatment) at 2, 6, and 10 wk after plant emergence. Samples not immediately processed were moistened and stored at 5°C. In the laboratory, roots were washed free of soil on a 2 mm sieve and shoots removed. Prior to assessing mycorrhizal infection, the root systems of the five plants from within each plot were subsequently

Table 1. Seeding rates of herbaceous plants used in the spoil tank study.

Plant Species	Amount of Seed Sown $(g \cdot m^{2})$	No. of Seeds · g ($\bar{X}$ + S.E.)
Slender Wheatgrass	2.3	263 ± 1
Alsike Clover	0.4	1333 ± 2
Sainfoin	16.4	37 ± 1

pooled and cut into 1 to 2 cm segments. The entire amount of root obtained per plot was used to determine the amount and status of the VAM infection.

Plants were sampled the second and fourth year toward the end of the growing season (i.e. late August). Since it was impractical to excavate whole intact root systems after the first year, two random soil samples (14 cm deep x 6 cm diam.) were taken using a hand trowel from each plot (six replicates per treatment). Roots were obtained from each sample by washing the spoil through 2 mm and 500 µm sieves. Roots present on the 2 mm sieve were removed, blotted, weighed, and kept for quantification of VAM infection. To obtain the fresh weight of root in the 500 µm fraction, the organic material was first separated from the heavier inorganic component through successive decantings. This material was subsequently dispersed in water and roots removed, blotted, and weighed. If there was too much organic debris in the 500 µm fraction for easy separation of the root material, a random subsample (10%) was obtained and the weight of root in the subsample was used to estimate the weight of root in the fraction. This value was added to the weight of root from the 2 mm sieve to obtain the total wet weight of root in the soil sample. A random (10%) of the wet weight of root was used to estimate the amount and status of the VAM infection.

A modification of the procedure described by Phillips and Hayman (1970) was used to observe the mycorrhizal infection. Roots were cleared in simmering 10% KOH for 6 min, rinsed in distilled water for 5 min, acidified in 0.1 N HCl for 5 min, and stained in simmering 0.01% trypan blue. These changes were necessary in order to reduce fragmentation of the roots and to decrease background staining.

VAM infection was quantified using a modification of the line intersect technique (Newman 1966). An occular grid (11 x 11 lines, 60 µm between lines) was used to estimate root length (cm) rather than using a single line. Giovannetti and Mosse (1980) have shown that a grid system provides for a more accurate measurement. Prior to quantification, roots were placed within a 2.4 x 5.0 cm area on a microscope slide. Care was taken to place as much root as possible in this area without having roots overlaying each other. This procedure was repeated until all the roots from a given sample were placed on microscope slides. Twenty five fields were examined per slide under a compound microscope at 160X magnification. Prior testing using filaments of known length showed that 25 fields per 2.4 x 5.0 cm area gave accurate, consistent estimates of filament length. For each slide, five kinds of measurements were made: length of root, length of mycorrhizal root, and length of mycorrhizal root containing either arbuscules, vesicles, or only hyphae. Although more than one type of mycorrhizal structure may be intersected by a grid line in any given field, only one structure was enumerated. The order of importance was arbuscules, vesicles and then hyphae.

Root length data from the first year samples were expressed on a per plant basis after dividing the total number of observations per sample by the number of plants sampled (five). The total lengths of root in each sample from the 2nd and 4th growing seasons were calculated from the total fresh wt of root and expressed as length of root (cm) per 10 cm^3 of spoil. Percent infection was determined by dividing total mycorrhizal root length by total root length. The necessity for expressing mycorrhizal infection levels on a root length basis rather than as a percentage of root length has been addressed by several investigators (e.g. Gerdemann 1968; Ambler and Young 1977).

2.3 QUANTIFICATION OF VAM FUNGAL SPORES AND SPOROCARPS

Spoil samples were taken from each slender wheatgrass plot on the amended oil sands tailing and subalpine coal mine spoil at the end of the third growing season (i.e. August 29, 1979) to estimate spore and sporocarp numbers of VA fungi. Three random soil samples to a depth of 10 cm were taken per plot, nine replicates per treatment. A modification (Smith and Skipper 1979) of the wet sieving and decanting technique described by Gerdemann and Nicolson (1963) was used to remove spores and sporocarps from the amended mine spoils. A 25 g (wet wt) subsample of spoil was placed in 200 ml of water and stirred for 5 min before pouring the material through a series of sieves (500, 250, 125, and 53 µm). The subalpine mine spoil samples were first passed through a 4 mm mesh sieve to remove large rock fragments and other debris. Material remaining on each sieve after washing with tap water for 3 min was transferred into separate 150 ml beakers to which 100 ml of water was added and vigorously stirred. Heavy material was briefly allowed to settle (30 s) and spores collected by pouring the water onto a 6 cm diam. filter paper held in a Millipore filter holder which was under suction. The material remaining in each beaker was then extracted again.

A modification (Smith and Skipper 1979) of the sucrose centrifugation method (Allen et al. 1979) was used to collect VAM fungal spores and sporocarps in the peat-amended spoils due to interference from organic matter. Ten grams (wet wt) of peat-amended spoil were wet sieved and the material remaining on each sieve was washed into separate 50 ml centrifuge tubes and made up to 35 ml with distilled water. Following centrifugation (2000 rpm, 10 min) the supernatant was filtered as above. The pellet remaining after the first centrifugation was resuspended in 2 M sucrose solution to bring the volume up to 35 ml., vigorously stirred, and centrifuged at 2000 rpm for 10 min; the supernatant was filtered. More than one filter paper had to be used per centrifuge tube to avoid masking of the spores by suspended fine particulate matter. Spores and sporocarps on the filter papers were counted at a constant magnification (25 or 50X) under a dissecting microscope. Spores that were cracked or heavily pitted were not included in the count. Numbers of spores and sporocarps per VA fungal species were expressed per 10 g-dry-wt of spoil. A representative number of each spore and sporocarp type were mounted in lactophenol on slides for identification. Taxonomy of the VAM fungi followed Gerdemann and Trappe (1974) and Hall and Fish (1979).

2.4 THE OCCURRENCE OF THE VA FUNGI IN THE PEAT AMENDMENT

Samples were collected in June 1980 from the peat deposit near Canmore, Alberta, which provided the material used in the tank study, to determine if VA mycorrhizal inoculum was present in this amendment. The peat deposit was forested with mature white spruce and had a dense undergrowth of shrubs and herbs. Portions of the deposit had been mined previously and used as an amendment on nearby coal mine spoils. Three profiles were sampled around the perimeter of the excavated site. The face of each profile was cleaned and samples were taken at the 20 to 30, 50 to 60, and 80 to 90 cm depths from each of the profiles. All peat samples were stored at 5°C until they could be broken up by hand and thoroughly mixed. Extraction and quantification of VAM fungal spores were as previously described for the peat-amended mine spoils.

To determine if viable inoculum was present in the peat, three pregerminated slender wheatgrass seeds were planted in 13 cm pots containing peat from each depth and profile and grown in the greenhouse for 10 wk. Plants were watered once at 3 wk with a 20-20-20 fertilizer solution (0.5 g · 4 L) to alleviate phosphorus deficiencies. At 10 wk, roots were washed free of the peat on a 10 mm sieve, blotted dry, and their fresh weight determined. A random 10% of the total fresh weight of roots in each pot was subsequently cleared, stained, and assessed for VA mycorrhizal as previously described.

2.5 PRIMARY PRODUCTION

Five slender wheatgrass plants from each plot per treatment were obtained at 10 wk after plant emergence to determine shoot weight per plant. Shoots were separated from roots and dried at 80°C to constant weight. Shoot production values (g dry wt · m^2) for the second and third growing season were estimated at the time of sampling for assessment of VA infection and VA fungal spore and sporocarp numbers by clipping three random, 25 x 25 cm quadrats per plot (i.e. nine replicates per treatment). The soil samples obtained for mycorrhizal evaluation the second year were taken from the center of the first two quadrats. Shoot material was dried at 80°C to constant weight.

2.6 CHEMICAL ANALYSIS

In June, 1977 (prior to planting) and after two growing seasons (September, 1978), soil samples were taken from the 0 to 5 cm depth in all amended and control plots for chemical analysis. Two random samples were taken per plot (three plots per treatment) and pooled. All samples were air dried and sieved through a 2 mm sieve. The following parameters were measured: NO^3-N, extractable P, DTPA extractable Pb and Zn and soil pH. Levels of NO^3-N were estimated by a phenol-disulphonic acid colorimetric procedure with a solution of copper and silver sulfate as the extractant. Extractable P was determined by a colorimetric measurement with molybdenum blue using ascorbic acid as the reductant following extraction with a sulfuric acid and ammonium fluoride solution. Amounts of Pb and Zn were measured by atomic absorption spectroscopy. The pH was determined after saturating the spoil with water. Chemical analyses and pH determinations were conducted as described by McKeague (1976). Additional information on the physical and chemical characteristics of the mine spoils and amendments can be found in Visser *et al*. (in prep.)

2.7 STATISTICAL ANALYSIS

Mycorrhizal and nonmycorrhizal root lengths, percent infection, spore and sporocarp numbers, shoot production, and nutrient analysis data, were statistically analyzed using one-way analyses of variance (Sokal and Rohlf 1969). Data sets underwent either ln, ln (x + 1), square root, or arcsine$\sqrt{p}$ transformation to render the variances homogenous as determined by the Bartlett's test. Several data sets were not tranformable and were analyzed using non-parametric tests. Details of these statistical tests are presented as footnotes to the appropriate tables.

3. RESULTS

3.1 VESICULAR-ARBUSCULAR MYCORRHIZAL DEVELOPMENT

3.1.1 Oil Sands

Vesicular-arbuscular mycorrhizal development during the first growing season was limited mainly to plants grown on the peat-amended plots (Table 2). There was some mycorrhizal infection in plants grown on the control plots at 10 wk after plant emergence, but the infection was very light and sporadic. VAM infection was first detected in plants grown on the peat-amended spoil at 2 wk (Table 2). Infection at this time consisted solely of hyphae (Table 3).

Infection levels at the end of the second growing season were higher ($p = 0.05$) in plants grown on the peat-amended plots as compared with plants on the other treatments (Table 4). VA mycorrhizae were observed for the first time in roots from the fertilized plots at the end of the second growing season, though at very low levels and sporadic in occurrence. VAM infection was not detected in roots from the sewage-amended plots.

After four growing seasons, infection levels were still highest ($p = 0.05$) in plants on the peat-amended spoil (Table 5). A low level of infection was detected for the first time in plants grown on the sewage-amended plots.

There were significant changes in the mycorrhizal status of slender wheatgrass plants between the second and fourth growing seasons (Table 6). The length of mycorrhizal root per 10 cm^3 of peat-amended spoil decreased ($p = 0.05$) during this period of time. Mycorrhizal root lengths and percent infection increased ($p = 0.05$) in the fertilized plots over the 2 yr period. The majority of plants in the control plots did not survive after 2 yr. However percent infection in the surviving plants increased from 4 to 36% ($p = 0.05$).

3.1.2 Subalpine Coal Mine

Vesicular-arbuscular mycorrhizal infection was detected at 2 wk after plant emergence only in plants grown on the peat-amended

Table 2. Initial VA-mycorrhizal development of slender wheatgrass grown on the amended oil sands spoil.[x]

	Time from Plant Emergence (weeks)	Treatment			
		Control	Peat	Fertilizer	Sewage
Mycorrhizal Root Length per Plant (cm)[y]	2	0	0.7	0	0
	6	0	6	0	0
	10	0.5[a]	37[b]	0	0
% Infection[z]	2	0	0.6	0	0
	6	0	4	0	0
	10	0.9[a]	23[b]	0	0

---

[x] Within a row, all non-zero means superscripted differently differ at $p = 0.05$, as indicated by Scheffé confidence intervals (Neter and Wasserman 1974).

[y] Data underwent $\ln(x + 1)$ transformation.

[z] Data underwent $\arcsin\sqrt{p}$ transformation.

Table 3. Status of the mycorrhizal infection (length of root which contained either arbuscules, hyphae, or vesicles) during the initial growth of slender wheatgrass on the amended oil sands spoil.[x]

Infection Status	Time from Plant Emergence (weeks)	Length of Root (cm) After the Following Treatments			
		Control	Peat	Fertilize	Sewage
Arbuscules	2	0	0	0	0
	6	0	3	0	0
	10	0	5	0	0
Hyphae	2	0	0.7	0	0
	6	0	4	0	0
	10	0.5^{a}	30^{b}	0	0
Vesicles	2	0	0	0	0
	6	0	< 0.1	0	0
	10	0	2	0	0

[x] Means superscripted differently, differ at $p = 0.05$ as indicated by Scheffé confidence interval. Data underwent $\ln(x + 1)$ transformation.

Table 4. VA-mycorhizal status of slender wheatgrass grown on the oil sands spoil, 2 years after application of surface amendments.[x]

	Treatment			
	Control	Peat	Fertilizer	Sewage
Mycorrhizal Root Length (cm · 10cm^3)[y]				
Total	0.6^a	130^b	1.2^a	0
Arbuscules	0.1^a	26^b	0.5^a	0
Hyphae	0.4^a	98^b	0.7^a	0
Vesicles	0.1^a	6^b	0.0	0
% Infection[z]	4.0^a	46^b	1.0^a	0

[x] Within a row all non-zero means superscripted differently differ at $p = 0.05$ as indicated by Scheffe confidence intervals.

[y] Data underwent ln (x + 1) transformation.

[z] Data underwent arcsine p transformation.

Table 5. VA-mycorrhizal status of slender wheatgrass grown on the oil sands spoil, 4 years after application of surface amendments.[w,x]

	Treatment			
	Control	Peat	Fertilizer	Sewage
Mycorrhizal Root Length (cm · 10cm^3)[y]				
Total	ND	51^a	10^b	1^c
Arbuscules	ND	9^a	2^b	0
Hyphae	ND	41^a	7^b	1^c
Vesicles	ND	1	1	0
% Infection[z]	36^a	62^b	43^c	0.3^d

[w] Within a row all non-zero means superscripted differently differ at p = 0.05 as indicated by Scheffe confidence intervals.

[x] There were too few plants in the control to estimate root lengths. Two plants were dug from each plot to determine percent infection.

[y] Data underwent ln (x + 1) transformation.

[z] Data was statistically analyzed using the non-parametric Friedman test.

Table 6. Comparison of the mycorrhizal status of slender wheatgrass after 2 and 4 years on the amended oil sands spoil.[x,y,z]

Treatment		Year	
		Second	Fourth
Control	% Infection	4	36*
Peat	Mycorrhizal Root Length (cm · 10cm^3)	130	51*
	% Infection	46	62
Fertilizer	Mycorrhizal Root Length (cm · 10cm^3)	1	10*
	% Infection	0.6	43*
Sewage	Mycorrhizal Root Length (cm · 10cm^3)	0	1
	% Infection	0	0.3

[x] Within a row, * indicates significant differences at $p = 0.05$ as indicated by a t-test.

[y] Root length data underwent square root transformation.

[z] % infection data underwent arcsine $\sqrt{p}$ transformation.

spoil (Table 7). Mycorrhizae were detected in plants grown on the control and fertilizer-amended plots at 6 wk after plant emergence. Infection was not detected in plants grown on the sewage-amended spoil until 10 wk. At 10 wk, there were no significant differences in total length of mycorrhizal root per plant among the amendments, although plants on the amended plots had more mycorrhizal roots than plants on the control ($p = 0.05$). The infection in plants grown on the peat-amended plots consisted solely of hyphae at the 2 wk sampling time (Table 8). While arbuscules were observed in roots of plants grown on the control and peat-amended plots at 6 wk, they were not observed in the roots of plants grown on the fertilizer- and sewage-treated plots until 10 wk after plant emergence.

Mycorrhizal root lengths after 2 years were highest ($p = 0.05$) in the peat-amended spoil as compared with the control and sewage-amended plots (Table 9). Mycorrhizal root lengths in the fertilized plots were not significantly different from the control, peat- or sewage-amended spoil. The status of the infection was also affected by the type of amendment applied. Lengths of root containing arbuscules were highest ($p = 0.05$) in the peat-amended spoil. Plants on the sewage-amended spoil had the lowest ($p = 0.05$) arbuscular root length. There were no significant differences in lengths of root with hyphae or vesicles among any of the treatments. Percent infection was suppressed ($p = 0.05$) in the sewage-treated plots compared with the percentages of infected roots from the control and peat-amended spoil.

Infection levels were still highest ($p = 0.05$) in the peat-amended spoil after four growing seasons (Table 10), than in the control or sewage-amended plots. Mycorrhizal roots lengths in the fertilized spoil were not significantly different than those in the control and peat-amended plots. The effects of the amendments on the status of the infection was similar to that observed after the 2nd year. Arbuscular root length was lowest ($p = 0.05$) in the sewage-treated spoil. However, the lengths of root which contained arbuscules were not significantly different among the other

Table 7. Initial VA-mycorrhizal development of slender wheatgrass grown on the amended subalpine coal mine spoil.[x]

	Time from Plant Emergence (weeks)	Treatment			
		Control	Peat	Fertilizer	Sewage
Mycorrhizal Root Length per Plant (cm)[y]	2	0	2	0	0
	6	3^a	13^a	1^a	0
	10	19^a	97^b	160^b	99^b
% Infection[z]	2	0	5	0	0
	6	4^a	10^a	0.5^a	0
	10	23^a	58^b	44^b	16^b

[x] Within a row, all non-zero means superscripted differently differ at p = 0.05, as indicated by Scheffé confidence intervals.

[y] Data underwent ln (x + 1) transformation.

[z] Data underwent arcsine$\sqrt{p}$ transformation.

Table 8. Status of the mycorrhizal infection (length of root which contained either arbuscules, hyphae, or vesicles) during the initial growth of slender wheatgrass on the subalpine coal mine spoil.[x]

Infection Status	Time from Plant Emergence (weeks)	Length of Root (cm) After the Following Treatments			
		Control	Peat	Fertilizer	Sewage
Arbuscules	2	0	0	0	0
	6	0.3^{a}	5^{b}	0	0
	10	8^{a}	40^{b}	66^{b}	31^{ab}
Hyphae	2	0	2	0	0
	6	3^{a}	8^{a}	1^{a}	0
	10	11^{a}	57^{b}	86^{b}	68^{b}
Vesicles	2	0	0	0	0
	6	0	0.2	0	0
	10	0	0.7^{a}	7^{a}	0.7^{a}

[x] Within a row, all non-zero means superscripted differently, differ at $p = 0.05$ as indicated by Scheffé confidence intervals. Data underwent $\ln(x + 1)$ transformation.

Table 9. VA-mycorrhizal status of slender wheatgrass grown on the subalpine coal mine spoil, 2 years after application of surface amendments.[x]

	Treatment			
	Control	Peat	Fertilizer	Sewage
Mycorrhizal Root Length (cm · 10cm^3)[y]				
Total	17^{a}	90^{b}	32^{ab}	24^{a}
Arbuscules	12^{a}	54^{b}	11^{a}	1^{c}
Hyphae	5^{a}	35^{a}	20^{a}	20^{a}
Vesicles	0.2^{a}	1^{a}	1^{a}	3^{a}
% Infection[z]	42^{a}	41^{a}	29^{ab}	9^{b}

[x] Within a row values superscripted differently differ at $p = 0.05$ as indicated by Scheffé confidence intervals.

[y] Data underwent $\ln (x + 1)$ transformation.

[z] Data underwent arcsine $\sqrt{p}$ transformation.

Table 10. VA-mycorrhizal status of slender wheatgrass grown on the subalpine coal mine spoil, 4 years after application of surface amendments.[x]

	Treatment			
	Control	Peat	Fertilizer	Sewage
Mycorrhizal Root Length (cm · 10cm^3)[y]				
Total	19^{ab}	64^{c}	45^{bc}	12^{a}
Arbuscules	14^{a}	12^{a}	28^{a}	5^{b}
Hyphae	4^{a}	48^{b}	15^{c}	7^{ac}
Vesicles	0.5^{a}	4^{b}	2^{ab}	0.2^{a}
% Infection[z]	70^{a}	51^{b}	70^{a}	32^{c}

[x] Within a row values superscripted differently differ at $p = 0.05$ as indicated by Scheffé confidence intervals.

[y] Data underwent $\ln(x + 1)$ transformation.

[z] Data underwent $\arcsin\sqrt{p}$ transformation.

treatments. Percent infection was highest (p = 0.05) in the control and fertilizer-amended plots and lowest in the sewage-treated spoil.

There were no significant changes in mycorrhizal root lengths from any treatment between the second and fourth growing seasons (Table 11). However, percent infection did increase (p = 0.05) in the control, fertilizer- and sewage-amended plots over the two years.

3.2 ROOT LENGTHS

3.2.1 Oil Sands

Root length of slender wheatgrass over the first growing season, was affected by treatment ($p \leq 0.05$) (Table 12). Root lengths per plant were greater (p = 0.05) for those grown on the fertilizer- and sewage-amended spoil than for plants grown on the control plots. Root lengths of plants grown on the peat-amended spoil were not significantly different from root lengths of plants grown on the other treatments. Across all treatments root lengths were highest (p = 0.05) at 10 wk after plant emergence.

Root lengths at the end of the second growing season (Table 13) were greater (p = 0.05) in the amended plots than in the control. However, there were no significant differences in root lengths among the amendments. After four growing seasons, (Table 13) root lengths were greater in the sewage-amended plots than in the fertilized ones (p = 0.05). Root lengths in the peat-amended plots were not significantly different from lengths in the fertilizer- or sewage-amended spoil. Between 2 and 4 years, root lengths decreased (p = 0.05) in the peat- and fertilizer-amended spoil. There were no significant changes in the sewage-amended plots.

3.2.2 Subalpine Coal Mine

There was a significant ($p \leq 0.01$) interaction between time and treatment on root lengths (cm) of slender wheatgrass during the first growing season on the subalpine coal mine spoil (Table 12). Root lengths in the control plots were not significantly different

Table 11. Comparison of the mycorrhizal status of slender wheatgrass after 2 and 4 years on the subalpine coal mine spoil.[x,y,z]

		Year	
		Second	Fourth
Control	Mycorrhizal Root Length (cm · 10cm^3)	17	19
	% Infection	42	70*
Peat	Mycorrhizal Root Length (cm · 10cm^3)	90	64
	% Infection	41	51
Fertilizer	Mycorrhizal Root Length (cm · 10cm^3)	32	45
	% Infection	29	70*
Sewage	Mycorrhizal Root Length (cm · 10cm^3)	24	12
	% Infection	9	32*

[x] Within a row, * indicates significant differences at $p = 0.05$ as indicated by a t-test.

[y] Root length data underwent ln transformation.

[z] Percent infection data underwent arcsine$\sqrt{p}$ transformation.

Table 12. Total root lengths (cm · plant) of slender wheatgrass during the first growing season on the amended mine spoils.

Spoil Type	Time from Plant Emergence (weeks)	Treatment				Significance Level[x]		
		Control	Peat	Fertilizer	Sewage	Time	Treatment	Interaction
Oil Sands[y]	2	88	108	99	99	*	***	NS
	6	58	145	180	302			
	10	63	164	543	353			
Subalpine[z]	2	32	47	28	35	***	**	**
	6	113	123	192	203			
	10	98	166	349	586			

[x] Significance Level: * $0.05 \leq p > 0.01$; ** $0.01 \leq p > 0.001$; *** $p \leq 0.001$; NS nonsignificant.

[y] Data were found to be nontransformable and were statistically analyzed using a non-parametric test of interactions (Marascuilo & McSweeny 1977). Effects of time and treatments were determined using the Friedman's test at $p = 0.05$.

[z] Data was analyzed using a two-way ANOVA after ln transformation.

Table 13. Total root lengths of slender wheatgrass on the amended mine spoils 2 and 4 years after application of surface amendments.[x,y]

Spoil Type	Years Following Amendation	Root Length ($\mathrm{cm} \cdot 10\mathrm{cm}^3$) Control	Peat	Fertilizer	Sewage
Oil Sands[z]	2	15^a	304^b	159^b	258^b
	4	ND	86^a	35^b	124^a
Subalpine	2	49^a	200^b	104^b	222^b
	4	26^a	125^b	65^{ab}	47^a

[x] Within a row, means superscripted differently differ at $p = 0.05$ as indicated by Scheffé confidence intervals.

[y] Data underwent ln transformation.

[z] There were too few plants in the control to estimate root lengths.

among the sampling times. Also, at the 2 and 6 wk sampling times, root lengths in the control plots were not significantly different from lengths in the amended plots. At 10 wk, root lengths in the amended plots were greater than in the control plots, but there were no significant differences among the amendments.

Root lengths of slender wheatgrass at the end of the second growing season (Table 13) were greater ($p = 0.05$) in the amended plots compared with the control. However, there were no significant differences in total root lengths among the amendments. After four growing seasons, root lengths were higher ($p = 0.05$) in the peat-amended spoil as compared with root lengths in the control and sewage-amended plots (Table 13). Root lengths in the fertilized spoil were not being significantly different from those in the other treatments. Root lengths did not change significantly between 2 and 4 years on the control, peat- or fertilizer-amended subalpine mine spoil. There was a decrease ($p = 0.05$) in the sewage-amended plots though during this time.

3.3 SHOOT PRODUCTION

3.3.1 Oil Sands

All amendments increased ($p = 0.05$) shoot production above the control during the first growing season (Table 14). Shoot weights were highest in the sewage-amended spoil.

Shoot production for the second growing season was again highest ($p = 0.05$) in the sewage-treated plots (Table 15). Peat and fertilizer also increased plant growth compared with the control. By the third year (Table 15), shoot production had decreased ($p = 0.05$) in all but the peat-amended plots compared with estimates made during the second growing season.

3.3.2 Subalpine Coal Mine

Shoot growth was highest ($p = 0.05$) during the first growing season in the fertilizer- and sewage-amended spoil

Table 14. Shoot weights of slender wheatgrass plants at the end of the first growing season on the amended mine spoils (10 wk after plant emergence).[x,y]

	mg Dry Wt · Plant			
Spoil Type	Control	Peat	Fertilizer	Sewage
Oil Sands	5^{a}	23^{b}	145^{c}	272^{d}
Subalpine	15^{a}	23^{a}	147^{b}	252^{b}

[x] Within a row, means superscripted differently differ at $p = 0.05$ as indicated by Scheffé confidence intervals.

[y] Data underwent ln transformation.

Table 15. Shoot production estimates of slender wheatgrass for the 2nd and 3rd growing seasons on the amended mine spoils.[x]

Spoil Type	Growing Season	$\text{g Dry Wt} \cdot m^2$ Control	Peat	Fertilizer	Sewage
Oil Sands[y]	2nd	2^{a}	110^{b}	167^{b}	766^{c}
Oil Sands[y]	3rd	0.02	80^{a}	18^{b}	50^{ab}
Subalpine[y]	2nd	43^{a}	111^{b}	210^{bc}	325^{c}
Subalpine[z]	3rd	27^{a}	102^{b}	45^{ab}	71^{ab}

[x] Within a row, means superscripted differently differ at $p = 0.05$ as determined by Scheffé confidence intervals.

[y] Data underwent ln transformation.

[z] Data underwent square root transformation.

(Table 14). Peat did not significantly increase plant growth the first year compared with the control.

Shoot production after two growing seasons (Table 15) was greater ($p = 0.05$) in all amendments than in the control. The highest production estimates were observed in the sewage-treated plots. Shoot production was significantly ($p = 0.05$) lower in the fertilizer and sewage-amended spoil after three years (Table 15) when compared with production estimates from the second growing seasons.

3.4 CHEMICAL ANALYSES OF THE MINE SPOILS

3.4.1 Oil Sands

Levels of extractable phosphorus in the amended oil sands spoil (0 to 5 cm) prior to planting were highest ($p = 0.05$) in the fertilized and sewage-amended plots (Table 16). Amounts of extractable P decreased ($p = 0.05$) over the first two years in the fertilizer- and peat-amended plots, and increased ($p = 0.05$) in the sewage-amended spoil (Table 16). There were no significant changes in P levels in the control.

All three amendments initially increased ($p = 0.05$) NO_3-N levels (0 to 5 cm) in the spoil, with the peat-amended plots having the highest ($p = 0.05$) levels (Table 16). Levels of NO_3-N in the fertilizer- and peat-amended spoil decreased ($p = 0.05$) over the two growing seasons. There were no significant changes in amounts of NO_3-N in the control and sewage-amended plots between the first and second growing seasons.

Initial levels of DTPA extractable Pb were not changed by amendation (Table 16). Zinc levels in the spoil were significantly increased ($p = 0.05$) by peat- or sewage-application compared with levels in the control and fertilized plots.

The pH of the spoil was very alkaline prior to the application of the surface amendments (Table 16). The addition of fertilizer and peat lowered the pH to neutrality. The addition of sewage also decreased pH of the spoil as compared with the control,

Table 16. Chemical analysis of the amended oil sands spoil (0 to 5 cm depth).[x]

		µg · g Dry Wt of Spoil				
Sampling Date	Treatment	Extractable[x] P	NO_3-N[x]	Pb[y]	Zn[y]	pH[z]
Prior to Planting	Control	$1^{a^{1}}$	$0.1^{a^{1}}$	0.6^{a}	0.3^{a}	8.7
(June, 1977)	Peat	$3^{b^{2}}$	$251^{b^{2}}$	3^{a}	11^{b}	7.3
	Fertilizer	$53^{c^{4}}$	$40^{c^{4}}$	1^{a}	0.6^{a}	7.0
	Sewage	$26^{c^{6}}$	$16^{c^{6}}$	3^{a}	3^{b}	7.8
September, 1978	Control	$2^{d^{1}}$	$0.8^{d^{1}}$			
	Peat	$0.8^{d^{3}}$	$62^{e^{3}}$			
	Fertilizer	$14^{e^{5}}$	$3^{d^{5}}$			
	Sewage	$169^{f^{7}}$	$7^{d^{7}}$			

[x] Data was statistically analyzed using 2-way ANOVAS following ln transformation. Letters compare treatments within one time, numbers compare time within one treatment at $p = 0.05$.

[y] Values not followed by the same letter differ at $p = 0.05$ as determined by a Scheffé confidence interval.

[z] Data was not statistically analyzed because of no variation amongst the replicates.

but the decrease was less than that observed for the other two amendments.

3.4.2 Subalpine Coal Mine

Levels of extractable P in the spoil (0-5 cm), prior to planting, (Table 17) were increased ($p = 0.05$) by the application of fertilizer or sewage. After two growing seasons, P levels were highest ($p = 0.05$) in the fertilizer- or sewage-amended spoil (Table 17). Amounts of extractable P in the peat-amended plots were lower ($p = 0.05$) than levels in the control plots. The levels of extractable P, within each treatment, did not change significantly over the first two growing seasons.

Prior to planting, NO_3-N levels were highest ($p = 0.05$) in the fertilizer- or peat-amended spoil (Table 17). After two growing seasons NO_3-N levels were still highest ($p = 0.05$) in the peat-amended spoil. There was a decrease ($p = 0.05$) in NO_3-N levels in the fertilized plots between the first and second growing season. Amounts of NO_3-N in the control, peat- or sewage-amended plots did not change significantly over the first two growing seasons.

Sewage amendation increased ($p = 0.05$) the amount of DTPA extractable Pb and Zn in the subalpine mine spoil (Table 17) prior to planting. Extractable Zn levels were also higher ($p = 0.05$) in the peat-amended plots than in the control.

The incorporation of peat into the spoil only resulted in slight changes in the pH of the material (Table 17). Fertilizer had no substantial effect on soil pH. However, sewage increased pH of the mine spoil.

3.5 VA FUNGAL SPECIES

Glomus aggregatum and *Glomus mosseae* were the most commonly encountered species of VA mycorrhizal fungi, occurring in all but the fertilizer- and sewage-amended oil sands plots (Table 18). The absence of VA fungi in these plots was consistent with the results obtained on mycorrhizal infection levels at the end of the second growing season. *Glomus aggregatum* has not been previously reported

Table 17. Chemical analysis of the amended subalpine coal mine spoil (0 to 5 cm depth).

		µg · g Dry Wt of Spoil				
Sampling Date	Treatment	Extractable^{x} P	NO_3-N^{x}	Pb^{y}	Zn^{y}	pH^{z}
Prior to Planting (June, 1977)	Control	3^{a1}	4^{a1}	0.5^{a}	0.9^{a}	7.5
	Peat	2^{a2}	449^{b2}	0.9^{a}	10^{b}	7.4
	Fertilizer	51^{b3}	150^{b3}	0.8	1^{a}	7.1
	Sewage	42^{b4}	25^{c5}	6^{b}	8^{b}	7.8
September, 1978	Control	5^{c1}	3^{d1}			
	Peat	0.3^{d2}	97^{e2}			
	Fertilizer	26^{e3}	4^{d4}			
	Sewage	89^{e4}	25^{f5}			

[x] Data was statistically analyzed using 2-way ANOVAS following ln transformation. Letters compare treatments within one time, numbers compare time within one treatment at p = 0.05.

[y] Values not followed by the same letter differ at p = 0.05 as determined by a Scheffe´ confidence interval.

[z] Data was not statistically analyzed because of no variation amongst the replicates.

Table 18. VA-mycorrhizal fungal species and numbers of spores and sporocarps present in the slender wheatgrass plots on the oil sands and subalpine amended mine spoils after three growing seasons.

Fugal Species	Spoil Type	Spore Numbers · 10 g Dry Wt. of Spoil Treatment Control	 Peat	 Fertilizer	 Sewage
Entrophospora infrequens (Hall) Ames & Schneider	subalpine	< 1	0	0	0
Glomus aggregatum Schenck & Smith	oil sands	3	182	0	0
	subalpine^x	52^a	180^b	19^{ac}	4
Glomus mosseae (Nicol & Gerd.)	oil sands	0.2	6	0	0
	subalpine^y	14^a	8^a	20^a	12^a
G. mosseae (sporocarps)	subalpine^x	21^a	0	18^{ab}	2^b

[x] Data underwent ln transformation. Values having a mean of zero were not included in the ANOVA. Means superscripted differently differ at $p = 0.05$ as indicated by Scheffe confidence intervals.

[y] Means are not significantly different at $p \leq 0.05$ as determined by post-hoc comparisons based on the nonparametric Krieskal-Wallis test.

from reclaimed mine spoils; having been found only under citrus (Schenck and Smith 1982). The spores of this species were also common in soil from a short-grass prairie southeast of Calgary and in the pasture lands and walkways around the spoil tanks. *Glomus mosseae*, the other commonly encountered endophyte has been reported from Western Canada (Molina *et al.* 1978) but not specifically from Alberta. Khan (1978) found *G. mosseae* in association with plants growing on coal tips in the Illawara region of New South Wales. A fifth species, *Glomus tenuis* (Green) Hall, with spores too small to be readily isolated from soil, was detected in the roots of slender wheatgrass grown on the peat-amended spoils during quantification of VAM infection levels by its characteristic hyphae and morphology of infection.

3.6 VA FUNGAL SPORE AND SPOROCARP NUMBERS

Spore numbers of *Glomus aggregatum* and *G. mosseae* in the oils sands spoil were highest ($p = 0.05$) in the peat-amended plots with low spore numbers in the control and no spores detected in the fertilizer- or sewage-amended spoil (Table 18).

Spore number of *G. aggregatum* in the subalpine spoil were highest ($p = 0.05$) in the peat-amended spoil followed by the control, with numbers the lowest in the sewage-amended plots. Numbers of spores in the fertilized plots were not significantly different from the values for the control or sewage-amended plots. There were no significant effects of amendation of the subalpine mine spoil on number of *G. mosseae* spores. Total spore numbers in the control and amended subalpine mine spoil were not significantly correlated with shoot production for the third growing season.

Sewage suppressed ($p = 0.05$) sporocarp production by *Glomus mosseae* in the subalpine mine spoil (Table 18). Fertilizer, however, had no effect on sporocarp numbers.

3.7 EFFECTS OF THE AMENDMENTS ON THE SIZE OF *GLOMUS MOSSEAE* SPORES AND SPOROCARPS

The numbers of spores and sporocarps of *Glomus mosseae* that were obtained on each sieve, after wet sieving the subalpine mine spoil, were used to examine the effects of the amendments on the size of these fungal propagules. *G. mosseae* was chosen since the range of spore and sporocarp sizes for this species (Gerdemann and Trappe, 1974) extended over the range of pore sizes used in the wet sieving procedure. There were an insufficient number of these propagules in the oilsands spoil to conduct a similar examination.

There were no substantial effects of fertilizer application to the subalpine mine spoil on the size class distribution of *G. mosseae* sporocarps compared with the control (Figure 2). Insufficient sporocarps were obtained from the peat- and sewage-amended plots to determine if these treatments had any effect on sporocarp size.

Single spores of *G. mosseae* obtained from the control and fertilized subalpine mine spoil occurred mainly within the 53 to 125 and 125 to 250 µm size classes (Figure 2). There were no significant differences in the numbers of spores occurring within these two size classes. A greater ($p = 0.05$) percentage of the total number of *G. mosseae* spores from the sewage-amended plots, however, occurred within the smallest size class. There were insufficient number of *G. mosseae* spores collected from the peat-amended spoil to examine the effects of this amendment on size class distribution.

3.8 THE OCCURRENCE OF VA FUNGI IN THE PEAT AMENDMENT

VA fungal spores were detected in samples from each of the sites in the peat deposit but their occurrence and numbers did not exhibit a consistent pattern with depth. While the majority of spores from site one and three were detected in the 50 to 60 cm depth, spores in site two occurred predominantly in the 20 to 30 cm layer.

Although VA fungal spores were detected from all the sites, they appeared to be nonviable. Spore walls were generally heavily

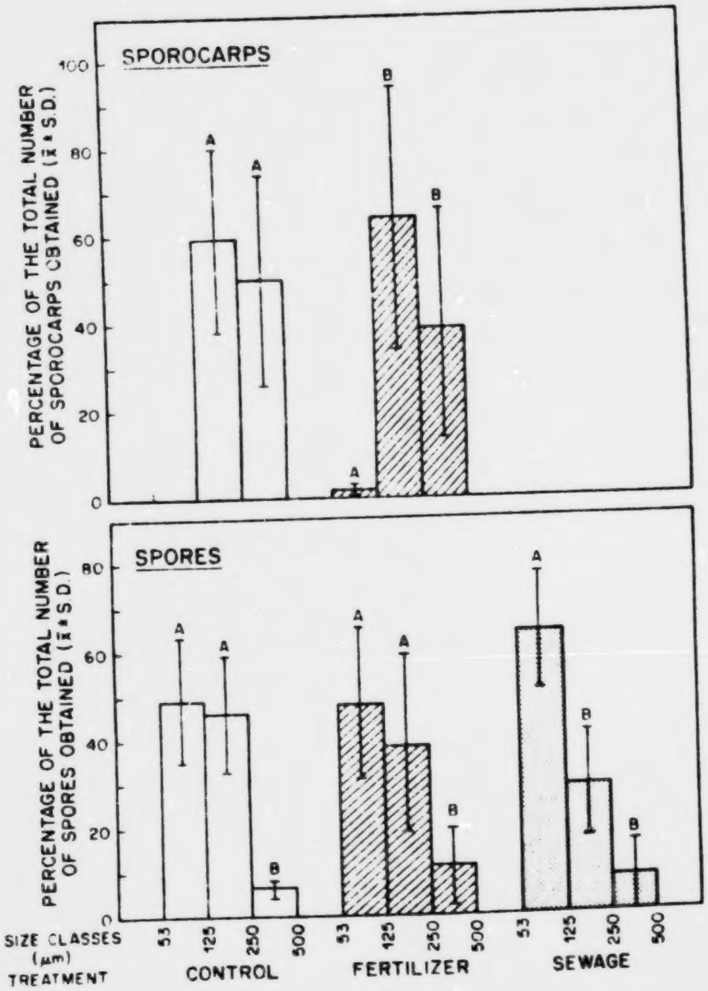

Figure 2. Size class distribution of *Glomus mosseae* sporocarps and spores obtained from the slender wheatgrass plots on the amended subalpine coal mine spoil at the end of the third growing season. Data underwent $\arcsin\sqrt{p}$ transformation to obtain homogeneous variances. For each treatment, the same letter above a bar indicates nonsignificant differences at p = 0.05 as indicated by Scheffé confidence intervals.

thickened and pitted and spores lacked content. The characteristics of these old spores suggested affinity with Glomus fasciculatum. However, chlamydospores found associated with the roots of slender wheatgrass from the bioassay study were identified as Glomus aggregatum.

The distribution of VA mycorrhizal inoculum within the peat deposit was patchy (Table 19) There was no apparent relationship between the presence of VA fungal spores and the subsequent mycorrhizal development of slender wheatgrass in the baiting study. The amount of VA-fungal infection, expressed either as mycorrhizal root length or as a percent of the total root length was variable and did not follow a trend with depth (Table 19).

Table 19. The distribution of viable vesicular-arbuscular mycorrhizal inoculum within the peat deposit using *Agropyron trachycaulum* as the host.[x]

	Profile 1			Profile 2			Profile 3		
Depth (cm)	20-30	50-60	80-90	20-30	50-60	80-90	20-30	50-60	80-90
Total Root Length (m)	81	127	32	45	52	30	22	175	7
Mycorrhizal Root Length (m)	0	0	0	12	20	23	0	123	0.3
% Infection	0	0	0	27	38	78	0	71	4

[x] Root length values represent the total amount of root produced by three plants grown in a 13 cm diam. pot under greenhouse conditions for 10 weeks.

4. DISCUSSION

4.1 INITIAL MYCORRHIZAL DEVELOPMENT

Initial rates of vesicular-arbuscular mycorrhizal development in slender wheatgrass grown on the oil sands and subalpine mine spoils were significantly affected by amendation. The type of amendment applied to a mine spoil and the rate of application can control the initial mycorrhizal development of plants used in revegetation programs by:

1. increasing VA fungal inoculum densities in the spoil,
2. altering soil nutrient levels and/or other chemical and physical properties.

The appearance of infection at 2 wk only in the peat-amended spoils and the presence of VA mycorrhizae mainly in plants on the peat-amended oil sands spoil indicated that the peat amendment had added VA fungal inoculum. This was confirmed in a later study. The rapid infection rates in the peat-amended soils presumably were indicative of infections being established from hyphae or infected root fragments (Hall 1976; Johnson 1977; Powell 1977; Hayman & Stovold 1979). The oil sands tailings were found, by wet sieving and decanting and by establishing slender wheatgrass plants on the spoil in the greenhouse, to be initially devoid of VA fungi.

The initial rates of VA mycorrhizal development in plants grown on the peat-amended spoils were similar to those reported by Ponder (1979) for ryegrass and sudangrass on a recently graded strip-mine spoil from Illinois and for plants grown on agricultural and undisturbed habitats (Sutton 1973; Read et al. 1976; Powell and Sithamparanathan 1977). The longer time until infection in plants from the control and fertilizer-amended subalpine mine spoil suggested that inoculum levels were considerably lower and/or that the inoculum was in a different form as compared with the peat-amended spoil.

The delay in infection in plants from the sewage-amended subalpine mine spoil may be due to factors other than inoculum levels. Spitko and Manning (1981) found that application of sewage sludge (4.7 and 9.4 t dry weight · per ha) to field plots initially inhibited the development of VA mycorrhizae. They suggested that the inhibition may have been the result of high levels of extractable P(222 to 269 ppm). In that initial extractable P levels were not significantly different between the fertilizer- and sewage-amended subalpine mine spoil, toxic substances(s), such as heavy metals, may have been responsible for the delay in infection in plants on the sewage-treated plots. Hepper and Smith (1976) showed that the addition of 0.7 ppm of Zn to culture media significantly reduced the germination of Glomus mosseae spores. While it is difficult to transpose laboratory results to field situation, the higher levels of heavy metals in the sewage-treated subalpine mine spoil than in the other treatments suggests that sensitivity of the VA fungal inoculum to heavy metals may have been one factor which contributed to the initial delay in infection. Zinc levels in the sewage-amended plots were not significantly different from levels in the peat-amended spoil, suggesting the Pb rather than Zn may be responsible for the suppression. However, the degree of sensitivity of the endophyte(s) in the peat- or sewage-amended plots to Zn levels could have been an ecotypic response. Since infection was initiated in plants grown on the sewage-amended plots between 6 and 10 wk after plant emergence, Pb and Zn levels may have been reduced over time to ineffective levels through absorption by the clay fraction, chelation with organic matter (Gaynor and Halstead 1976), and (or) immobilization in decomposer biomass.

Arbuscules have been shown to be the sites of P transfer between endophyte and host (Schoknecht and Hattingh 1976). The length of time until their appearance in roots of plants grown on spoils of low nutrient status may have significant effects on seedling survival. The length of root or more importantly the volume of root containing arbuscules that is necessary before the plant benefits from the symbiosis is unknown. Stribley et al. (1980) indicated that infection levels of less than 20% in onions did not appear to have an effect on shoot yield, but they gave no data on the proportion of the infection that was arbuscular. While slender wheatgrass on the peat-amended spoils contained arbuscules at 6 wk, it is unknown whether the infection was functional at this time.

4.2 VA FUNGAL INOCULUM

The ability of a habitat to recover to predisturbance conditions following mining activity and subsequent reclamation, will depend in part on the successful reestablishment of mycorrhizal relationships. The success and rate of reestablishment will be determined by the effects of reclamation practices on fungal species present and inoculum densities in the reclaimed site. Spores of mycorrhizal fungi may constitute an important source of infection in reclaimed mine spoils where plant densities over the short term are usually low. Total spore numbers from the control and amended subalpine coal mine spoil [spore numbers per 10 g-dry-wt of spoil X ± S.D., control (66 ± 62), peat (188 ± 115), fertilizer (39 ± 36) and sewage (16 ± 29)] were higher than spore numbers reported from coal mine wastes (Khan 1978) or from an agricultural field (Hayman *et al.* 1975) but were lower, except in the peat amended spoil, in comparison with numbers found by Sutton and Barron (1972) from agricultural fields in Ontario.

The application of sewage sludge to a mine spoil, by suppressing spore production, could significantly affect the success of reclamation programs by slowing the rate of VA fungal inoculum buildup in these sites. Spore production of *Glomus aggregatum* was significantly reduced in the sewage-amended subalpine coal spoil, 3 yr after the original application, compared with the other treatments. In mine spoils where VA inoculum levels are initially low or where mycorrhizal development is suppressed, nonmycorrhizal plant species, usually members of the Chenopodiaceae (Miller 1979) that colonize the site, could have a competitive advantage over mycorrhizal dependent species, especially if nutrient become limiting (Janos 1980). Over time, the composition of the site would change from mycorrhizal dependent to nonmycorrhizal species. Under these circumstances successional blocks may be created in the system, since nonmycorrhizal species do not provide for the buildup of VA fungal inoculum and subsequent species which require VA mycorrhizae for nutrient uptake and growth are excluded from the habitat (Reeves *et al*. 1979; Janos 1980). Allen and Allen (1980) reported that *Salsola kali*, a nonmycorrhizal plant species, predominated on strip-mine soils with less than 1 spore per g for 10 yr.

Sewage sludge application to the subalpine coal mine spoil not only resulted in decreased numbers of *G. aggregatum* chlamydospores but also affected the size of *G. mosseae* spores. Spore size, as a function of the amount of nutrient reserves, may be a major factor factor determining VA fungal spore longevity, and the ability of these propagules to germinate and establish successful infection. Garrett (1973) has commented that the average size of the reproductive propagule of a plant pathogenic fungus is determined by the nutritional requirements for establishment of a new individual of the species in its typical habitat. Garrett's statement suggests that spores of VA fungi which occur at the small end of the spore size range for the species would have a lower probability of causing infection than spores larger in size. Ross (1980) reported that germination of small (< 75 um) *Glomus macrocarpum* Tul. & Tul. var *geosporus* (Nicol. & Gerd.) Gerdemann & Trappe chlamydospores from soybean fields was less than that of larger (> 105 um) ones.

4.3 VA MYCORRHIZAL DEVELOPMENT: 2ND AND 4TH YEAR

The low levels and sporadic nature of the VAM infection in the control and fertilized oil sands tailings during the second year suggested that the oil sands spoil, originally devoid of mycorrhizal inoculum, was being colonized by VA fungi, though at very low rates. There is some question as to the means by which VA fungal inoculum is dispersed and the distance over which effective dispersal can occur. Movement of inoculum either in the form of spores or root fragments can only be accomplished by the physical movement of soil particles (Gerdemann and Trappe 1974). McIlveen and Cole (1976) showed that worms, ants, and birds could be agents of dispersal for VA fungal spores by their movement of soil particles. It is doubtful whether this type of dispersal is a major contributor to long distance transport and would likely be more important to within habitat movement of inoculum. The distance from the mine site to potential VA fungal inoculum pools and the rates of dispersal and subsequent recolonization would likely be important factors that need to be considered in the planning of reclamation programs. Success or failure of a revegetation strategy may be determined in part by the immigration rates of VA fungi into these disturbed habitats. This would be especially true if the spoil lacked mycorrhizal inoculum prior to reclamation, such as the oil sands tailings, and inoculum was not introduced either via an amendment or by planting mycorrhizal seedlings.

The decision to use a specific amendment or one application rate versus another could have significant consequences on the outcome of a revegetation program. The peat amendment consistently resulted in higher levels of infection in both spoils compared with sewage application, which suppressed mycorrhizal development, although more severly in the oil sands tailings than on the subalpine coal mine spoil. Because of the nutrient poor condition of most mine spoils, the addition of some form of nutrients will be necessary to achieve maximum plant growth even if mycorrhizae are present. Hughes et al. (1978) and Menge et al. (1978) showed that the addition of nutrients to mycorrhizal plants increased growth above levels obtained for mycorrhizae alone. Slender wheatgrass plants on the controls exhibited less total root lengths than plants on the amended spoils even though mycorrhizal root lengths may not have been significantly different. Consideration should be given to choosing an amendment application regime that will not only ensure optimum plant growth and survival but which will not retard or suppress mycorrhizal development. The initial absence of mycorrhizal inoculum coupled with high soil P levels could have been the primary factor responsible for the suppression of infection in plants on the sewage-amended oil sands spoil. The reduced infection levels of plants on the sewage-amended subalpine coal spoil may have resulted solely from high levels of soil P.

The effects of various reclamation practices on VA mycorrhizal development of plants used in a revegetation program may depend on the VA fungal inoculum potential on the site prior to revegetation, the species of endophytes present, and the abiotic characteristics (e.g. pH, nutrient content, moisture retention) of the spoil. While slender wheatgrass on the fertilizer- and sewage-amended subalpine coal mine spoil were infected by the end of the first growing season, plants on the fertilized- and sewage-treated oil sands tailings did not become mycorrhizal until the end of the second and fourth growing seasons respectively. These differences in mycorrhizal response to fertilizer- or sewage, between the two spoil types may have been due to the lack of inoculum in the oil sands spoil.

The lack of significant changes in the mycorrhizal status of slender wheatgrass on the subalpine coal spoil, over the four years, indicates that initial reclamation procedures will have long term effects on VA mycorrhizal development. Allen and Allen (1980) also found that plants on topsoil amended mine spoils in Wyoming were only 50% of infection levels in plants from undisturbed habitats after 3 yr. By influencing mycorrhizal development, the type of amendment applied to mine spoil could determine the rate of recovery and the success of a reclamation program.

4.4 PLANT GROWTH

Since mycorrhizal and nonmycorrhizal plants did not occur on the same treatment, it is difficult to ascertain whether VA infection was necessary for the growth of slender wheatgrass on the controls and amended spoils. Over the short term, the absence of infection in plants grown of the sewage-amended oil sands spoil and the low infection levels in the sewage-amended subalpine coal mine spoil had no apparent effect on plant growth. It is interesting however, that shoot production peaked in the fertilizer- and sewage-amended plots on both mine soils during the second growing season and was significantly lower the 3rd year, but not in the peat-amended plots where plants were heavily mycorrhizal. These results suggest that while high nutrient applications may result in greater short term primary productivity, the subsequent suppression of mycorrhizal development, as a result of the high nutrient levels, may lead to wide fluctuations in plant growth. Mycorrhizal relationships may be important in maintaining stability in primary production from year to year.

5. CONCLUSIONS

1. The type of amendments applied to the mine spoils controlled the initial rates of VAM development in slender wheatgrass by increasing fungal inoculum densities in the spoils and by altering soil nutrient levels and/or other chemical and physical properties.

2. The peat used in this study was found to contain VA fungal inoculum. The rapid infection rates in the peat-amended spoils presumably were indicative of infections being established from hyphae or infected root fragments.

3. The initial application of the amendments had long term effects on the development of VA mycorrhizal relationships in these disturbed soils. Slender wheatgrass plants on the fertilizer- and sewage-amended oil sands tailing took 2 and 4 yr respectively to develop VA mycorrhizae. There was no significant change in the mycorrhizal status of plants on the subalpine coal spoil over the 4 yr period. In general, changes in the mycorrhizal status of plants on mine spoils may occur only slowly with time.

4. The rate of dispersal of VAM inoculum appears to be very low. The distance from the mine site to potential VA fungal inoculum pools and the rates of inoculum dispersal and subsequent recolonization are important factors that need to be considered in the planning of a reclamation program.

5. *Glomus aggregatum* and *Glomus mosseae* were the dominant endophytes in the two mine spoils. Spore numbers and occurrence of these species were influenced by the amendment and the type of mine spoil.

6. Sewage significantly decreased spore production of *G. aggregatum* and the size of *G. mosseae* spores in the subalpine coal spoil. By suppressing Va fungal spore production and by affecting the size of spores produced by an endophyte, the application of sewage to a mine spoil may significantly alter the outcome of the revegetation program by slowing the rate of inoculum buildup within the spoil.

6. RECOMMENDATIONS

1. If the rapid reestablishment of VAM infection in plants grown on mine spoils is to be a major concern of a reclamation strategy, attention will have to be given to determining: a) initial Va fungal inoculum densities in the mine spoils, b) application rates of amendments which do not suppress mycorrhizal development but allow for optimum plant growth and survival. The effects of specific amendments on mycorrhizal development will depend on the nutrient status of the mine spoil and the VA fungal species and spore densities in the mine soil prior to reclamation.

2. The determination of nutrient response curves for mycorrhizal plants of species to be used in a revegetation program would be important in determining the optimum levels of fertilizer application.

3. Where disturbance is severe enough to either eliminate or significantly reduce VA fungal inoculum levels in a mine spoil, such as the oil sands, inoculum must be added to the site. The rate of natural recolonization by VAM fungi appears to be too slow to allow for their rapid reestablishment in these sites. The addition of inoculum can be accomplished by the addition of an amendment which contains VA fungi, such as the peat used in this study, or through the preinoculation of transplanted seedlings.

4. The use of commercially produced inoculum for large scale field inoculations is not feasible at this time. Research is needed not only to develop techniques for the introduction of inoculum prior to seeding but for ensuring adequate infection from the introduced inoculum.

5. Further research should be directed toward obtaining information of VA fungal species occurrences in mine spoils, their ecological tolerance limits, the effects of nutrient addition, and VA fungal species efficiency in promoting plant growth and survival.

6. Further information is needed on the effects of sewage sludge on VAM development before recommendations can be made concerning its use in a reclamation program. Future research should examine: a) the effects of toxic compounds in sewage on VA spore

germination and mycorrhizal development, b) application of different levels of sewage on mycorrhizal development.

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REINSTATEMENT OF BIOLOGICAL ACTIVITY IN SEVERELY DISTURBED SOILS: ECTOMYCORRHIZAE IN AMENDED OIL SAND TAILINGS AND SUBALPINE COAL MINE SPOIL AND IN UNDISTURBED JACK PINE AND SPRUCE STANDS

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Abstract

The mycorrhizal status of jack pine and bearberry grown in oil sand tailings treated with various amendments (peat, mineral fertilizer, liquid sewage sludge) and of white spruce and willow grown in subalpine coal mine spoil using the same amendments was monitored for 4 years. In addition, ectomycorrhizae associated with jack pine and white spruce on undisturbed and disturbed field sites were studied and compared to those found in the amendment study.

Application of sewage sludge increased the rate of ectomycorrhizal development of jack pine, slowed the mycorrhizal development of white spruce and bearberry, and apparently did not affect the mycorrhizae of willow. Peat introduced inoculum of several species of ectomycorrhizal symbionts and when ectomycorrhizal inoculum in the spoil was low, its application resulted in more rapid mycorrhizal development. Mineral fertilizer had little effect on mycorrhizal development. The most common ectomycorrhizal fungus was the E-strain which dominated white spruce in all treatments and jack pine in the peat. At the end of 4 years the E-strain fungi were being replaced on white spruce by Amphinema byssoides and was apparently replacing Thelephora terrestris on jack pine in all treatments except the peat amendment. Agarics constituted a minor portion of the ectomycorrhizal fungi whereas at least four Ascomycetes and three Aphyllophoreales were present. Once established, the specific symbioses were stable for at least several years.

More than 50 species of ectomycorrhizal fungi were identified from fruit bodies on a mature jack pine field site. On this site Cenococcum geophilum, Tricholoma flavovirens and Lactarius spp. formed a large proportion of the ectomycorrhizae. Species fruiting in an adjacent cutline were largely different from those occurring in the undisturbed mature stand. Amphinema byssoides was a dominant symbiont of both mature spruce trees and of spruce seedlings regenerating on roadcuts.

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1. INTRODUCTION

Mycorrhizal associations are present in the vast majority of vascular plants under natural conditions. Although many of these normally mycorrhizal plants can survive and even thrive without fungal symbionts under artificial conditions, it is generally accepted that mycorrhizae are essential for long term success in the field. Host plants may benefit from mycorrhizal associations in several ways. Foremost among these is enhanced nutrient uptake, especially P with endomycorrhizae but other nutrients as well with ectomycorrhizae. It has been proposed that ectomycorrhizae enhance water uptake and increase drought tolerance (Trappe, 1977) but definitive experimental evidence is still lacking to support this view. Mycorrhizal symbioses may also increase the tolerance of higher plants to other adverse conditions. Certain ectomycorrhizae can increase the tolerance of plants to high soil temperatures (Marx et al., 1970) and extremes of soil pH (Clement et al., 1977), offer protection from root pathogens (Marx, 1973) and perhaps increase plant tolerance to toxic metals (Benson et al., 1980).

Mine spoils and tailings can be generally characterized as adverse environments for plant growth. They are usually infertile, low in organic matter, possess small nutrient reserves, have low moisture-holding capacities, are subject to excessive leaching, erosion and high soil temperatures and, depending on the particular spoil, they may have unfavorable chemical characteristics (Jurgensen, 1979). Thus vegetation on spoils may be subjected to a variety of stresses that might be at least partially alleviated by mycorrhizal infection. Of primary importance is an increase in efficiency of water and nutrient uptake and the conservation of the nutrient pool. Mycorrhizae may function in conserving nutrients indirectly by stimulation of plant growth and incorporation of nutrients into biomass or directly by more efficient exploitation of the soil and interception of nutrients by extramatrical mycelium that might be leached away in coarse textured spoils.

For these reasons it was felt that mycorrhizal symbioses were a critical component in the establishment of biological activity in mine spoils and tailings and worthy of detailed studies. The objectives of the ectomycorrhizal studies were to (1) monitor the development of ectomycorrhizae of woody plant species in a subalpine coal mine spoil and extracted oil sand tailings, (2) determine how potentially operational amendation procedures would affect rates of ectomycorrhizal development and the fungal symbionts involved, (3) determine the stability of ectomycorrhizal associations of a 4 year period (observations on fruit body production for 5 years), and (4) determine the ectomycorrhizal fungi occurring in natural stands of jack pine and white spruce. It should be emphasized that it was not the intention of these studies to determine the effects of ectomycorrhizae on plant growth, nor to determine the relative merits of particular fungal-host combinations.

In order to facilitate the monitoring of ectomycorrhizal development, spoil from a subalpine coal mine and tailings from an oil sand extraction plant were transported to Calgary, placed in large wooden tanks and planted with plant species that were likely candidates for use in the reclamation of each mine waste. This allowed frequent observations and maintenance of the experimental plots.

2. TANK STUDIES

2.1 MATERIALS AND METHODS

2.1.1 Tank Set-up

The subalpine coal mine spoil was from a site near Luscar, Alberta forested with *Picea glauca* (Moench) Voss, *Picea engelmannii* Parry, *Picea* hybrids and *Abies lasiocarpa* (Hook.) Nutt. prior to mining. The spoil was transported to Calgary, deposited in large wood-enclosed tanks (Figure 1), amended, and the amendments

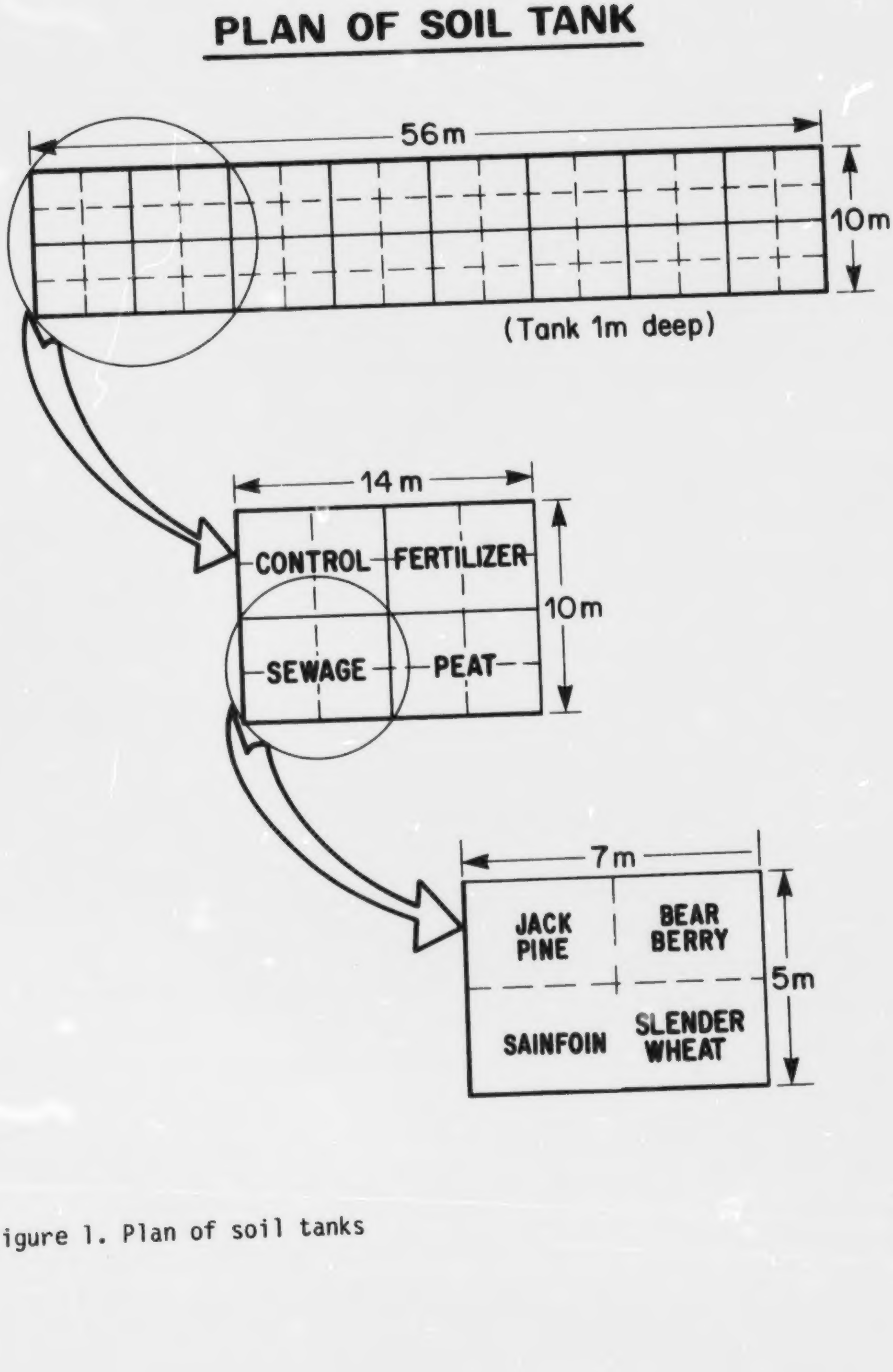
PLAN OF SOIL TANK
56m
10m
(Tank 1m deep)
14 m
CONTROL
FERTILIZER
SEWAGE
PEAT
10m
7m
JACK PINE
BEAR BERRY
SAINFOIN
SLENDER WHEAT
5m

Figure 1. Plan of soil tanks

rototilled into the spoil. The amendments consisted of (1) none, (2) a 14 cm deep-layer of feather moss peat collected from a mature white spruce forest near Canmore, Alberta, (3) mineral fertilizer equivalent to 113 kg N, 113 kg P_2O_5 and 91 kg K_2O ha^{-1}, and (4) anaerobically digested liquid sewage sludge applied at a rate of 46 mT dry weight ha^{-1}. Each treatment was replicated three times.

The oil sand tailings were obtained from the Great Canadian Oil Sands plant near Fort McMurray, Alberta. The tailings consisted almost entirely of fine-grained sand, was extremely low in organic matter and virtually sterile. The tailings were transported to Calgary and amended in the same manner as was the subalpine spoil. The characteristics of the coal mine spoil and the oil sand tailings are given in Table 1. Additional details of the amendments are reported by Visser et al. (1984).

2.1.2 Planting and Harvesting for Primary Production Estimates

White spruce seedlings were grown under greenhouse conditions in 192 cm^3 Spencer-Lamaire containers (Spencer-Lamaire Industries Ltd., Edmonton, Alberta) containing commercial, unsterilized peat moss. The seedlings were planted in the subalpine spoil at the age of 20 weeks using 20 cm spacing. Laurl leaf willow (*Salix glauca* L.) was commercially grown from cuttings in 95 cm^3 plastic containers filled with peat moss. The willow was planted in the subalpine spoil using 25 cm spacing. Survival was determined each spring by counting all individual plants and accounting for plants destructively sampled. Winter injury and subsequent dieback of the willow following the third winter was estimated in June by randomly selecting five individuals from each of the three replicate plots and measuring total height and total live height.

Jack pine (*Pinus banksiana* Lamb.) seedlings were grown in the greenhouse in 47 cm^3 Spencer-Lamaire book-containers by the staff of the Northern Forest Research Centre, Edmonton, Alberta. The seedlings were planted in the oil sand tailings when 20 weeks old with 20 cm spacing. Common bearberry (*Arctostaphylos uva-ursi*

Table 1. Chemical characteristics of a subalpine mine spoil and oil sand tailings following amendation with either peat, sewage sludge or fertilizer, or left unamended.

Spoil Type	Treatment	Total N (%)	NO_3-N ($\mu g.g^{-1}$)	Available P ($\mu g/g^{-1}$)	pH
Subalpine	Control	0.1	4	3	7.1
	Peat	1.6	449	1	7.0
	Fertilizer	0.1	150	51	6.7
	Sewage	0.1	25	42	6.9
Oil Sands	Control	0	0	1	7.3
	Peat	0.8	251	3	7.1
	Fertilizer	0.1	40	53	6.4
	Sewage	0.1	16	26	6.8

(L.) Spreng.) were purchased from a commercial nursery and were grown from cuttings in 100 cm^3 plastic pots filled with a peat-sand mixture. The bearberry cuttings were planted in the tailings with 20 cm spacing. Survival was determined in the same manner as for the subalpine woody plants. Winter injury of bearberry was estimated by randomly selecting five individuals from each replicate plot and visually estimating the amount of each plant that was killed.

Plants were harvested in September and early October in successive years to determine dry matter production. Five randomly selected individuals from each plot were used except in the fourth year when only three spruce were removed from each plot. Willow was not sampled after the third year due to browsing by deer, winter injury, and the assumption that the root systems extended below the spoil material into the subsoil. All samples were dried at 80°C to a constant weight. Leaf and needle samples were ground in a Wiley mill and analyzed for nutrient content using methods described by McKeague (1976).

2.1.3 Mycorrhizal Assessments

2.1.3.1 Sampling and mycorrhizal counts. Mycorrhizae of all plant species were sampled in September and October by digging up three randomly selected plants from each plot. A flat spade was used to remove all the soil midway between adjacent plants to a depth of 15 cm. Loose soil was shaken off the roots in the field and the three root systems from each plot were pooled and stored in plastic bags at 5°C until they could be processed. Only roots extending beyond the planting plug were analyzed for mycorrhizae.

All active short roots in each subsample were counted and evaluated. Short roots were considered to be active and alive if (1) the meristem, if visible, was pale and turgid, (2) the short root was turgid but not necessarily terete, and (3) if dark coloured and the meristem not apparent, the cortex was torn open with fine forceps and if brown inside, it was considered to be dead. All live short roots in which the length/diameter ratio exceeded 1.5 were considered to be susceptible to ectomycorrhizal infection and were rated. Short roots were rated as positive if any Hartig net was present, regardless of mantle development. Short roots were frequently checked using whole mounts in which very early stages of infection could be detected. The whole mounts were examined using a 40x brightfield, oil immersion objective. In order to detect intracellular infections, hand-cut sections were made, mounted in 0.05% trypan blue, heated until steaming occurred and examined with phase-contrast and brightfield optics. Additional details of the mycorrhizal assessments are given in Appendix 1.

At the end of the first growing season, all of the short roots of the spruce seedlings were rated for infection. In the evaluations after the second and fourth growing seasons, 300 randomly selected short roots per plant (i.e. 900/plot, 2700/treatment) were rated as to ectomycorrhizal status. All distinctive ectomycorrhizal types were counted separately.

The degree of infection of willow at the end of the first year was so low and morphological differentiation so slight that a staining technique was used to detect infection. Willow roots from each plot were cut into 2 to 3 cm lengths and 20 randomly selected segments were cleared in hot 10% KOH for 30 min, rinsed in water for 5 min, rinsed again in 0.1 N HCl for 5 min, strained in hot .01% trypan blue for 5 min and stored in lactophenol (Phillips and Hayman, 1970). At the end of the second growing season, the spoil was very hard and dry and a majority of the feeder roots were broken during sampling and thus unsuitable for ectomycorrhizal determinations. The actual number of feeder roots rated ranged from 40 to 300 per plot rather than the 900 desired. The roots were not cleared and stained at this sampling time.

Bearberry roots that extended beyond the planting plug were rated for mycorrhizal infection, dried at 80^{0}C and weighed. At the end of the first growing season, three plants in each of three replicate plots were randomly selected, the root systems combined and the total number of short roots counted and the mycorrhizal status rated. After the second growing season, each of the nine plants per treatment were evaluated separately by rating 300 short roots per plant.

At the end of the first growing season, three jack pine seedlings from each plot were randomly selected, the roots extending beyond the planting plug cut off and pooled, and the total number of short roots counted and evaluated. At the end of the second and fourth growing seasons, the roots from each of nine individual seedlings per treatment were evaluated separately. Three-hundred short roots from each seedling were counted. In addition, each sample was recounted to determine the total number of short root tips and the number of short roots that had been broken off. All final counts were corrected for breakage with the assumption that the broken roots did not differ from intact roots. Root lengths were determined by direct measurements of all lateral roots (those roots exceeding 1 cm in length) that occurred outside the planting plug and by measuring 50 short roots per seedling.

2.1.3.2 *Characteristics of specific ectomycorrhizae*. The E-strain symbionts were identified using the criteria of Danielson (1982), the most important of which was the large size (4 to 8 μm) of the cells of the mantle. It could be distinguished from *Thelephora terrestris* (Ehrh.) Fr. and other Basidiomycetes by the nearly glabrous mantle in contrast to the presence of thin, hyaline hyphae on *T. terrestris* + jack pine ectomycorrhizae. It was not possible to recognize *T. terrestris* ectomycorrhizae with certainty without using cultural techniques.

Ectomycorrhizae were considered to be formed by *Cenococcum geophilum* Fr. if (1) the ectomycorrhizae were black, (2) stiff pigmented hyphae radiated out from the surface, (3) the hyphae were 4 to 6 m in diameter with simple septa, and (4) the mantle consisted of the hyphal arrangement illustrated by Trappe (1971).

Roots infected with Mycelium radicis atrovirens Melin (MRA) were not counted but could possibly be mistaken for C. geophilum ectomycorrhizal infections, as the short roots may become black with extensive development of MRA. However, MRA differs from C. geophilum by the more olivaceous colour of the extramatrical mycelium of MRA, and most importantly, hyphal diameters which do not exceed 4 μm.

I-type ectomycorrhizae were characterized by the presence of thin setoid crystidia on the mantle, thus fitting into the I-subtype of Dominik (1962). Cystidia may be absent on some ectomycorrhizae of the I-type, but even if absent, these ectomycorrhizae can still be identified by the textura epidermoidea (Eckblad, 1968) hyphal structure and similarity to adjacent cystidial forms. The septa of the cystidia and young mantle cells possessed Woronin bodies.

Amphinema byssoides ectomycorrhizae were characterized by (1) the abundance of cream-coloured extramatrical hyphae, (2) pale yellowish mycelial strands, (3) hyphae 2 to 3 μm in diameter with large keyhole type clamps, and (4) mycelium which became dark-yellow in KOH. Culturing was required to confirm the identity of A. byssoides.

What is referred to as Suillus ectomycorrhizae was characterized by (1) dichotomous to subtuberculate form, (2) abundant extramatrical mycelium, (3) conspicuous mycelial strands, (4) colours varying from white to vinaceous, (5) mycelium covered with crystalline material and/or resinous deposits and simple septa, (6) a colour change to vinaceous or purple-brown of some of the excreted material on the hyphae when placed in 3% KOH. These characters would also apply to ectomycorrhizae formed with certain Rhizopogon species but for convenience, such ectomycorrhizae were assumed to be formed by Suillus species.

2.1.3.3 Direct isolation techniques. Direct isolation techniques were used to quantify and identify symbionts that did not produce distinctive ectomycorrhizae. For spruce, the entire root systems of three plants from each plot was cut into 2 to 3 cm segments and 12 segments from each plot were randomly selected and given 15 2-min wash cycles in an automatic root washing machine (Bissett and Widden, 1972). At the end of the second year, 10 washed segments from each plot were randomly selected and the first three mycorrhizae from one end were plated directly on benomyl-MMN agar (Appendix 1). At the end of the fourth growing season, the roots were sampled and washed in the same manner, but 12 root segments were selected and the first five mycorrhizae were plated on benomyl-MMN agar resulting in a total of 60 mycorrhizae plated per plot (180 per treatment). In addition, washed roots were surface sterilized and plated on MMN+ agar (Appendix 1) to isolate Ascomycete symbionts. Root segments were dipped in 95% ethanol, soaked in 30% H_2O_2 for 15 sec, rinsed in two changes of ice-cold sterile water for 1 h and plated, five/plate, on MMN+. Ice water was used for rinsing off the H_2O_2 in hopes of stopping the action of the H_2O_2 more quickly than with water at room temperature (Appendix Tables 2 and 3). Representative isolates were subcultured on MMN and PDA for identification and grouping.

Attempts to isolate the symbionts from the bearberry mycorrhizae were made in the second year by plating 30 mycorrhizae per seedling directly on benomyl-MMN and by plating 20 tips per seedling on the same medium after being given 15 2-min washes.

Isolation procedures for jack pine were similar to those used with white spruce. The combination of surface sterilization and benomyl-MMN agar resulted in the failure to recover any probable symbionts after the first growing season. Thus, after the second growing season, washed ectomycorrhizae were plated directly on benomyl-MMN and after the fourth year, surface sterilization was combined with plating on MMN+ agar to detect Ascomycetes. Isolations were made from jack pine ectomycorrhizae within the planting cores in the second year to obtain estimates on the symbionts that were introduced with the seedlings. Fungi were identified by matching cultures with those obtained from fruit bodies. Specimens and cultures of all symbiont species are deposited at the Biosystematic Research Institute (DAOM), Agriculture Canada, Ottawa.

2.2 RESULTS FOR THE SUBALPINE COAL MINE SPOIL

2.2.1 Primary Production of White Spruce and Willow

The survival of both willow and spruce was high for the first two growing seasons and amendation had no effect (Table 2). Willow was subject to winter injury and dieback of stems which was particularly severe in the peat amended spoil after the third winter. Subsequent observations indicated that winter injury occurred in all amendments and that the total height of willow was limited by stem dieback.

The shoot growth of white spruce was not significantly affected by amendation until the fourth growing season (Table 3). Growth in the sewage and peat amended spoil was significantly greater than in the fertilizer amended spoil. Fourth year seedlings in the control treatment were notably paler green than in the sewage or peat treatments, indicating N deficiency. In contrast to white spruce, willow showed responses to the amendments every year it was sampled (Table 4). Sewage sludge resulted in an average 10-fold increase in shoot weight after three seasons as compared to the unamended spoil, whereas peat and fertilizer resulted in 3- to 4-fold increases. The growth of willow in the fertilizer treatment was highly variable.

2.2.2 Foliar Analysis

Amendation had no effect on concentrations of K, Ca, Mg, Mn or Zn in spruce needles after one growing season (Table 5). Phosphorus levels of needles were significantly higher in the control than in the peat and fertilizer treatments. Iron and Cu levels of needles were lower in the peat than in the other three treatments.

Table 2. Survival of white spruce and survival and height dieback of willow grown in subalpine coal mine spoil that was amended with either peat, sewage sludge or fertilizer, or left unamended[1].

Amendment	Spruce		Willow		Percent height dieback of willow
	Number of Growing Seasons				
	1	2	1	2	
	% Survival				
Control	93^a	90^a	95^a	91^a	7^a
Peat	92^a	88^a	95^a	92^a	60^b
Fertilizer	93^a	89^a	97^a	94^a	12^a
Sewage	89^a	84^a	92^a	91^a	8^a

[1] Data within each column analysed by 1-way ANOVA after data arcsin p transformed. Where F-test was significant differences in means was determined by Tukey pairwise comparisons. Numbers within each column superscripted by the same letter are not signifcantly different ($p = .05$).

Table 3. Shoot weight of white spruce grown in subalpine coal mine spoil that was amended either with peat, fertilizer or sewage sludge, or left unamended.[1]

Amendment	Number of Growing Seasons			
	1	2	3	4
	Shoot dry weight (g)			
Control	0.3^{a}	1.5^{a}	6.1^{a}	13.8^{ab}
Peat	0.3^{a}	1.3^{a}	6.9^{a}	21.4^{b}
Fertilizer	0.4^{a}	1.7^{a}	5.6^{a}	10.8^{a}
Sewage	0.4^{a}	2.3^{a}	9.4^{a}	21.2^{b}

---

[1] Data within each column analysed by 1-way ANOVA. Numbers superscripted with the same letter within each column do not differ significantly at p = .05, as determined by Scheffé multiple contrasts where F-test significant.

Table 4. Shoot weight of willow grown in subalpine coal mine spoil that was amended either with peat, fertilizer or sewage sludge, or left unamended.[1]

Amendment	Number of Growing Seasons		
	1	2	3
	Shoot dry weight (g)		
Control	0.6^{a}	4.5^{a}	14.4^{a}
Peat	0.9^{a}	13.7^{b}	41.1^{b}
Fertilizer	2.3^{c}	11.1^{b}	62.0^{ab}
Sewage	3.0^{c}	38.8^{c}	151.8^{c}

[1] Data within each column analysed by 1-way ANOVA. Numbers superscripted with the same letter within each column do not differ significantly at p = .05, as determined by Scheffé multiple contrasts where F-test significant.

Table 5. Elemental concentrations of white spruce needles after the plants were grown for one growing season in subalpine coal mine spoil either amended with peat, fertilizer or sewage, or left unamended.

Amendment	P	K	Ca	Mg	Mn	Fe	Zn	Cu
				$\mu g.g^{-1}$				
Control	2778^{b}	5627^{a}	12295^{a}	2175^{a}	2015^{a}	423^{b}	48^{a}	9^{b}
Peat	2085^{a}	4765^{a}	13314^{a}	2374^{a}	1541^{a}	116^{a}	41^{a}	2^{a}
Fertilizer	2207^{a}	5130^{a}	13208^{a}	2462^{a}	1600^{a}	546^{b}	40^{a}	7^{b}
Sewage	2562^{ab}	5102^{a}	12575^{a}	2549^{a}	1966^{a}	574^{b}	50^{a}	8^{b}

[1] Data within each column analyzed by one-way ANOVA. Scheffé multiple contrasts (p = .05) used to locate significant differences. Values in each column superscripted by the same letter to not differ significantly.

Nitrogen concentrations were not measured in spruce needles due to the large sample size required but foliar N of the willow after 2 years was significantly higher in the peat amended spoil than in the control (unamended) spoil.

2.2.3 White Spruce Ectomycorrhizae

Initially sewage sludge had a suppressive effect on ectomycorrhizae formation of white spruce (Table 6). However, by the end of the second growing season there were no differences in percent short roots that were ectomycorrhizal of plants from the variously amended spoil. Overall ectomycorrhizal infection rates increased significantly ($p = .001$) between 1 and 2, and 2 and 4 years. After 4 years, nonmycorrhizal short roots were rare. On all occasions, a majority of the ectomycorrhizae were considered to be active. As determined by direct counts, the E-strain was the dominant ectomycorrhizal symbiont at the end of the fourth growing season (Table 6). The E-strain fungi were not evaluated in previous years as criteria for identification had not been developed. *Cenococcum geophilum* was the only other ectomycorrhizal fungus that could be recognized by direct observation. It was not detected in the first year, occurred at low levels in all treatments except the peat at the end of the second year, and had almost disappeared by the fourth year.

On benomyl-MMN agar, which is selective for Basidiomycetes, *Amphinema byssoides* was isolated from 15 ± 21% ($x \pm SD$) of the ectomycorrhizae in all four treatments in the fall of the fourth year (Table 7). Variation was such that no significant differences could be detected among treatments (i.e., types of amendment). There was also no significant differences ($p = .05$) in isolation frequency of *A. byssoides* between 2 and 4 years. However, in that *A. byssoides* occurred in 8 of the 12 samples (all treatments considered) in the fourth year and was not detected in the second year, it is certain that it was increasing in abundance. It was also readily detected by direct observation in the fourth year,

Table 6. Ectomycorrhizal development of white spruce seedlings planted in subalpine coal mine spoil which was either amended with peat, fertilizer or sewage sludge, or left unamended.

	All Fungi[1]			E-strain[2]	Cenococcum geophilum[2]		
	Number of growing seasons[3]						
Amendment	1	2	4	4	1	2	4
Control	74^{a}	93^{a}	100^{a}	80^{a}	0	1.5^{ab}	0
Peat	51^{a}	88^{a}	99^{a}	60^{a}	0	0^{a}	0
Fertilizer	66^{a}	96^{a}	99^{a}	94^{a}	0	1.3^{ab}	0
Sewage	1^{b}	91^{a}	100^{a}	48^{a}	0	3.6^{b}	0.3

[1] Data analysed by 2-way ANOVA after 2 arcsin p transformation. Numbers within the total infection group superscripted by the same letter are not significantly different (p = .05) as determined by Scheffé multiple contrasts.

[2] Data analysed by 1-way ANOVA. Numbers within the same column superscripted by the same letter are not significantly different (p = .05) as determined by Scheffé multiple contrasts where F-test significant.

[3] At the end of first growing season, all short roots on the sample plants were evaluated for ectomycorrhizal status, the actual number ranging from 91 to 471 per seedling, with a mean of 261. After the second and forth growing seasons, 300 short roots per seedling were evaluated.

Table 7. Percent isolation frequency of two symbionts of white spruce (*Amphinema byssoides* and *Tomentella* sp.) and nonmycorrhizal fungi from ectomycorrhizae grown in amended and nonamended subalpine coal mine spoil for 2 or 4 years.[1]

	Amphinema byssoides[2]		*Tomentella* sp.		Nonmycorrhizal fungi[3]		No growth of any fungi[3]	
Amendment	2 yr	4 yr	2 yr	4 yr	2 yr	4 yr	2 yr	4 yr
	% isolation frequency							
Control	0	5^{a}	0	0	28^{b}	7^{a}	72^{a}	88^{a}
Peat	0	15^{a}	8	4	27^{b}	1^{a}	66^{a}	81^{a}
Fertilizer	0	7^{a}	0	0	19^{b}	5^{a}	81^{a}	88^{a}
Sewage	0	35^{a}	0	0	40^{b}	5^{a}	60^{a}	60^{a}

---

[1] For the second year, 30 mycorrhizae were plated/plot; for the fourth year, 60 mycorrhizae/plot were plated. All nonsurface sterilized and plated on benomyl-MMN agar.

[2] Data within 4 year column analysed by 1-way ANOVA. Values within column superscripted with the same letter do not differ significantly at p = .05 as determined by Scheffé multiple contrasts where F-test significant.

[3] Data analysed by 2-way ANOVA. Values within a group (nonmycorrhizal or no growth) superscripted by the same letter do not differ significantly (p = .05) as determined by Scheffé multiple contrasts where F-test significant.

whereas all the ectomycorrhizae observed in the second year appeared to be the E-strain type. Yellow mycelial strands and floccose mycelium typical of A. byssoides were common in the fourth year but were never observed previously. After 4 years, some ectomycorrhizae were observed to be infected with the E-strain at the base and with a clamped Basidiomycete (presumably A. byssoides) at the tip. It thus appeared that A. byssoides was able to replace the E-strain after the E-strain was established on short roots.

The only other ectomycorrhizal fungus isolated on benomyl-MMN agar was a Basidiomycete, Tomentella sp., which occurred only in the peat amended spoil at low levels. This species may produce ectomycorrhizae with cystidia (R.M. Danielson, unpublished data) but none were seen on the white spruce mycorrhizae. However, cultures from white spruce matched those obtained from cystidoid jack pine ectomycorrhizae. There were no differences in the ectomycorrhizae yielding no growth of any fungai either among amendments or between years. It is obvious that the major symbiont(s) was not capable of growth on the benomyl-MMN medium.

An average of 9 ± 14% (x ± SD) of the ectomycorrhizae from all four treatments plated on MMN+ after surface sterilization yielded Amphinema byssoides (Table 8). The major symbiont isolated was the E-strain which grew from 44 ± 21% (x ± SD) of the mycorrhizae plated from all treatments. The low percentage of E-strain isolated from ectomycorrhizae from the peat treatment may have been due to the inability of the E-strain fungi indigenous to the peat to grow on MMN agar. The growth of nonmycorrhizal fungi from the root tips reduced the chance of detecting slow growing symbionts. Cylindrocarpon destructans (Zins.) Scholten, Myxotrichum sp., Mycelium radicis atrovirens (MRA) and a sterile dark form were the major nonmycorrhizal fungi that grew from surface sterilized mycorrhizae (Table 9).

Table 8. Frequency of isolation of three symbionts of white spruce (E-strain, *Amphinema byssoides* and *Tomentella* sp.) and nonmycorrhizal fungi from surface sterilized ectomycorrhizae grown in amended and nonamended subalpine coal mine spoil for 4 years.[1,2]

Amendment	E-strain	*Amphinema byssoides*	*Tomentella* sp.	Non-mycorrhizal fungi	Mycorrhizae yielding no growth of any fungi
		% isolation frequency			
Control	64^{a}	2^{a}	0	43^{ab}	6^{ab}
Peat	22^{a}	8^{a}	3	28^{a}	41^{b}
Fertilizer	46^{a}	3^{a}	0	54^{b}	13^{ab}
Sewage	44^{a}	25^{a}	0	54^{b}	1^{a}

[1] Sixty ectomycorrhizae were plated per plot on MMN+ agar.

[2] Data within each column analysed by 1-way ANOVA. Values in each column superscripted with the same letter do not differ significantly at p = .05 as determined by Scheffé multiple contrasts where F-test significant.

Table 9. Percent frequency of isolation of nonmycorrhizal fungi from surface sterilized white spruce mycorrhizae plated on MMN+ at the end of the fourth growing season. Seedlings planted in a subalpine coal mine spoil that was amended with either peat, fertilizer or sewage sludge, or left unamended.[1]

Taxa	Amendment			
	Control	Peat	Fertilizer	Sewage
Cylindrocarpon destructans	3^a	11^a	6^a	2^a
Myxotrichum sp.	7^a	7^a	19^a	11^a
Mycelium radicis atrovirens	0^a	1^a	7^a	7^a
Symbiont 2118	0^a	$<1^a$	0^a	0^a
Sterile dark 2013	13^a	$<1^a$	1^a	10^a
Ascomycete 1977	1^a	0^a	4^a	1^a

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[1] Data within each row analysed by 1-way ANOVA. Values in each row superscripted with the same letter do not differ significantly at $p = .05$ as determined by Scheffé multiple contrasts where F-test significant.

2.2.4 Willow Mycorrhizae

At the end of the first growing season, willow roots were ectomycorrhizal only in the peat treatment and then at a low rate (Table 10). The absence of ectomycorrhizae in three treatments in the first year suggest that all inoculum originated in the peat. In the stained roots from the first year, no VA mycorrhizae were detected but resting spores of Olpidium sp. were present in several samples. After 2 years growth, infection rates were uniform among treatments with a small percentage of the feeder roots infected with Cenococcum geophilum except in the peat amended spoil. Although symbionts other than C. geophilum were not quantified due to lack of distinctive features, Tomentella sp. and the E-strain fungi were observed to be present. In the fifth growing season, Hebeloma sp. fruited prolifically in all three of the willow plots amended with sewage and in one of the fertilizer amended plots. Tomentella sp. produced large diffuse, resupinate fruit bodies on the surface of the peat under the willow canopy. Hebeloma sp. could not be cultured but Tomentella sp. was successfully brought into pure culture.

2.3 DISCUSSION OF AMENDING SUBALPINE COAL MINE SPOIL

The relative values of peat, sewage sludge and fertilizer for enhancing primary production on the subalpine spoil depended upon the test plant. White spruce, a slow growing conifer, was a poor short-term indicator of changes in soil nutrient levels. Its slow growth rate would do little to prevent leaching losses of soluble nutrients from the spoil. In contrast, willow responded quickly to amendation and incorporated large quantities of nutrients in stems and leaf litter. Sewage sludge was clearly the best spoil amendment for primary production of willow, but peat was equally good for spruce.

Amendation had two major effects on ectomycorrhizal development of white spruce; a short-term inhibition of ectomycorrhizal formation by sewage and the introduction of some symbionts (Tomentella sp., A. byssoides, and possibly E-strain) in the

Table 10. Ectomycorrhizal development of willow planted in subalpine coal mine spoil which was either amended with peat, fertilizer or sewage sludge, or left unamended.[1]

Amendment	All fungi		*Cenococcum geophilum*	
	Number of growing seasons			
	1	2	1	2
Control	0	51^a	0	2^b
Peat	12	67^a	0	0^a
Fertilizer	0	65^a	0	1^{ab}
Sewage	0	63^a	0	1^{ab}

[1] Data for second growing season analysed by 1-way ANOVA. Values in each column superscripted with the same letter do not differ significantly at p = .05 as determined by Scheffe multiple contrasts where F-test significant.

peat. It would not seem that the temporary delay in ectomycorrhizal development had no significant effect on growth and survival of spruce seedlings.

The peat contained a non-chlamydospore producing taxon of the E-strain, which was characterized in culture by a slow growth rate, dark pigmentation and abundant stiff, ornamented hyphae. The major form of the E-strain indigenous to the spoil produced chlamydospores and was lighter in colour than the E-strain fungi from peat. The term E-strain refers to a species or complex of similar species that have Ascomycete affinities (Danielson, 1982). Information on geographic distribution and ecological preferences are limited in that it cannot be linked to a known teleomorph, but observations on ectomycorrhizae indicate that the E-strain is a common and widespread symbiont. With pine hosts the E-strain is very common under nursery conditions in both Europe and North America (Laiho, 1965; Mikola, 1965). Under forest conditions, E-strain associations with pine are considered to be rare, especially in mature forests (Laiho, 1965). However, Mikola (1965) did find E-strain ectomycorrhizae associated with seedlings growing in burned clearcut areas, situations in some ways like those of mine spoils. Pines infected with E-strain and transplanted to forest soils appear to experience a change of symbionts soon after outplanting (Mikola, 1965); this is unlike the relatively stable condition found in the subalpine spoil.

The success of the E-strain in the subalpine spoil may be due in part to low levels of inoculum of other symbionts, or it may be more competitive than Laiho (1965) suggests. The occurrence in undisturbed forests has been based largely on the intracellular infection of pine ectomycorrhizae and little is known about its association with other tree species in which the infection is restricted to intercellular positions (e.g. spruce). Ectomycorrhizal data from this study strongly suggests that large amounts of inoculum were present in both the subalpine spoil and the peat amendment. Both of these materials were from mature forests dominated by spruce and indicate that the E-strain was an active symbiont under forest conditions. It may be that the E-strain is a common symbiont of mature spruce or other conifers but not of pines except in highly disturbed systems.

The fungus that appeared to be replacing the E-strain was *Amphinema byssoides*. Recently, ectomycorrhizal isolates were matched with cultures obtained from fruit bodies, thus permitting identification (R.M. Danielson, unpublished data). *Amphinema byssoides* was only detected once after 2 years, but was the second most common symbiont after 4 years. It appears likely that *A. byssoides* will continue to replace the E-strain and eventually become dominant in all spoil treatments.

Amphinema byssoides appears to occur wherever spruce and pine occur, and very probably is one of the most common ectomycorrhizal fungi. Despite this, there is just one report of *A. byssoides* as an ectomycorrhizal fungus. Fassi and De Vecchi (1962) describe it as being common in pine nurseries in Italy. Ectomycorrhizae formed by *A. byssoides* are surrounded by extremely large amounts of extramatrical mycelium, and Fassi and De Vecchi (1962) suggest that loss of nutrients by leaching is reduced by direct uptake from decomposing litter. Whether *A. byssoides* is directly involved in the decomposition of litter remains to be determined but the presence of such large amounts of hyphae may indeed reduce leaching losses as compared to fungi which produce little external mycelium (e.g. E-strain).

Tomentella is another genus of resupinate fungi that has been considered to be entirely saprophytic (Larsen, 1968). At least some *Tomentella* species are closely related to members of the genus *Thelephora* and it is not unreasonable that the genus *Tomentella* may include ectomycorrhizal species. The *Tomentella* species associated with white spruce in this study was reaily cultured although Larsen (1968) states that species of *Tomentella* do not grow in culture. *Tomentella* sp. was observed fruiting on the surface of the peat beneath willow in the fifth growing season. Cultures were established from fruit bodies and matched with cultures from spruce ectomycorrhizae. Tomentella was introduced with the peat and it is of interest that a species indigenous to cool, wet organic soils has persisted in the mine spoil for over 4 years.

2.4 RESULTS FOR THE OIL SAND TAILINGS

2.4.1 Primary Production of Jack Pine and Bearberry

Sewage sludge was clearly the best amendment for the growth of jack pine seedlings (Table 11). Mineral fertilizer had a minimal and short-term effect on growth whereas the beneficial effect of peat was more long lasting. Browsing by deer during the fourth winter introduced additional variation and limited any further primary production measurements. Survival of jack pine was good in all treatments except the control (Table 12). Survival of bearberry was lower than jack pine and after the first winter was lowest in the high nutrient treatments. Extensive dieback of bearberry occurred during the second winter but it could not be related to the amendment treatments due to variation caused by differences in exposure to wind.

2.4.2 Development of Bearberry Mycorrhizae

Sewage suppressed mycorrhizal infection of bearberry for at least 2 years (Table 13). It is presumed that most of the inoculum was introduced with the rooted cuttings. Thelephora terrestris was observed fruiting on some containers but the actual abundance of mycorrhizae when planted is unknown. The suppression of mycorrhizal development may have been due to the inhibition of specific fungi as one species was frequently isolated from plants grown on all types of amended tailings except the sewage amendment (Table 14). The mycorrhizae were all monopodial in the sewage amended spoil, whereas most mycorrhizae in the other three amendments were cruciate or coralloid. All sections examined showed the anatomy of the mycorrhizae to be of the arbutoid type (Harley, 1969) with the infection limited to the first layer of cortical cells.

Table 11. Shoot weight of jack pine seedlings grown in oil sand tailings amended either with peat, fertilizer or sewage, or left unamended.[1]

Amendment	Number of growing seasons			
	1	2	1	2
	Shoot Dry Weight (g)			
Control	$.5^{a}$	$.6^{a}$	$.6^{a}$	1.2^{a}
Peat	1.1^{b}	2.6^{b}	7.7^{c}	5.8^{a}
Fertilizer	1.0^{ab}	2.6^{b}	2.4^{b}	3.1^{a}
Sewage	1.0^{b}	10.4^{c}	24.5^{d}	54.6^{b}

[1] Data for second growing season analysed by 1-way ANOVA. Values in each column superscripted with the same letter do not differ significantly at p = .05 as determined by Scheffé multiple contrasts where F-test significant.

Table 12. Survival of jack pine and bearberry and winter dieback of bearberry of plants grown on oil sand tailings amended with either peat, fertilizer or sewage sludge, or left unamended.

Amendment	Percent Survival				Percent dieback of bearberry
	Jack Pine		Bearberry		
	1 yr	2 yr	1 yr	2 yr	
Control	86^{a}	54^{a}	62^{a}	40^{ab}	20^{a}
Peat	92^{ab}	88^{ab}	74^{a}	58^{b}	17^{a}
Fertilizer	86^{ab}	80^{ab}	31^{b}	21^{a}	19^{a}
Sewage	98^{b}	97^{b}	38^{b}	30^{ab}	1^{a}

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[1] Data within each column analysed by 1-way ANOVA. Values in each column superscripted by same letter not significantly different (p = .05) as determined by Tukey pairwise comparisons where F-test significant. All but percent dieback data were 2 arcsin p transformed prior to analysis.

Table 13. Mycorrhizal development of bearberry plants grown for 1 and 2 years in oil sand tailings either amended with peat, fertilizer or sewage sludge, or left unamended.[1]

Number of growing seasons	Amendment			
	Control	Peat	Fertilizer	Sewage
	Percent of short roots mycorrhizal			
1	52^{a}	81^{a}	68^{a}	0.1^{b}
2	74^{a}	70^{ab}	79^{a}	24^{b}

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[1] Each year's data analysed with the same letter by 1-way ANOVA. Means superscripted in each row not significantly different ($p = .05$) as determined by Scheffé multiple contrasts where F-test significant.

Table 14. Percent frequency of isolation on benomyl-MMN of bearberry symbiont R-1444 after 2 years from oil sand tailings either amended with peat, fertilizer or sewage sludge, or left unamended.

Root Treatment	Amendment			
	Control	Peat	Fertilizer	Sewage
None	4	5	2	0
Washed	12	20	38	0

2.4.3 Development of Jack Pine Ectomycorrhizae

2.4.3.1 Direct counts and overall ectomycorrhizal infections. During the first year, ectomycorrhizal infection of jack pine short roots was very low with all amendments except the peat (Table 15). After the second growing season, infection levels increased in all treatments with levels in the peat being significantly ($p = .05$) greater than with the other three amendments. A large amount of variation existed among rates of ectomycorrhizal infection. The low rates were associated with small quantities of mycorrhizae (low inoculum potential) within the planting cores. When seedlings with low inoculum in the plugs were omitted from the data analysis, peat and sewage amendments did not differ from one another and they had significantly greater infection rates than plants in the control and fertilizer amended tailings. Cenococcum geophilum formed ectomycorrhizae with 10% of the short roots of one seedling.

Between the second and fourth years, the rates of ectomycorrhizal infection increased significantly ($p = .05$) in all types of amended tailings except the control. Rates of ectomycorrhizal infection differed significantly among all amendments in the fourth year. The increases of mean ectomycorrhizal infection with time were probably largely due to the decrease in variability by virtue of seedlings with low levels of inoculum in the planting core gradually becoming heavily ectomycorrhizal (Figure 2). Variability in the mycorrhizal status of the short roots decreased significantly ($p = .005$) between the second and fourth years in all amendment treatments except the fertilizer.

The largest root systems, in terms of weight, length, and number of short roots were found in the sewage amended spoil (Table 16). The lateral roots were 1.5 times as long as the total length of short roots with no significant differences among treatments. There appeared to be differences in the frequency of short root initiation and lateral root diameters after the second year

Table 15. Ectomycorrhizal development of jack pine seedlings grown in oil sand tailings either amended with peat, fertilizer or sewage sludge, or left unamended.[1]

Number of growing seasons	Amendment			
	Control	Peat	Fertilizer	Sewage
	Percent of short roots ectomycorrhizal (SD)			
1	7^{a} (5)	25^{a}(6)	5^{a} (2)	5^{a} (2)
2	33^{ab}(27)	72^{a}(26)	24^{b}(17)	49^{ab}(38)
4	29^{a} (19)	91^{d} (6)	60^{b}(11)	83^{c} (17)

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[1] Data analysed by 1-way ANOVA. Differences among means detected by Scheffé pairwise comparisons. Values in each row followed by the same letter to not differ significantly (p = .05).

Table 16. Characteristics of the root systems of jack pine seedlings after two seasons growth in oil sand tailings amended with either peat, fertilizer or sewage sludge, or left unamended.[1]

Root Characteristics[2]	Amendment			
	Control	Peat	Fertilizer	Sewage
Short roots mycorrhizal (%)	33.5^{ab}	72.2^{b}	24.5^{b}	49.4^{ab}
No. of short roots/seedling	1200^{a}	2236^{ab}	3103^{b}	7609^{c}
No. of tips/seedling	1436^{a}	2772^{ab}	3368^{b}	8746^{c}
Mean short root length (mm)	1.94^{ab}	2.08^{a}	1.55^{a}	2.34^{a}
Short root length/seedling (m)	2.18^{ab}	4.77^{a}	4.88^{b}	17.44^{c}
Lateral root length/seedling (m)	2.69^{a}	5.90^{b}	7.15^{b}	23.92^{c}
Total root length/seedling (m)	4.87^{a}	10.68^{b}	12.03^{b}	41.36^{c}
Root weight in core/seedling (g)	$.32^{a}$	$.79^{b}$	$.53^{c}$	1.61^{d}
Root weight in soil/seedling (g)	$.23^{a}$	$.69^{b}$	$.75^{b}$	2.82^{c}
Total root weight/seedling (g)	$.51^{a}$	1.48^{b}	1.27^{b}	4.43^{c}
No. short roots/cm lateral root	4.6^{a}	3.8^{ac}	4.4^{a}	3.2^{bc}
No. of tips/short root	1.3^{ac}	1.4^{b}	1.1^{a}	1.3^{bc}
Length of laterals/length short roots	1.34^{a}	1.54^{a}	1.76^{a}	1.39^{a}
Root length (cm)/root weight (mg)	2.22^{a}	1.67^{b}	1.62^{b}	1.48^{b}
Root length in soil (cm)/shoot weight (g)	747^{a}	364^{b}	612^{ac}	469^{bc}

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[1] Data within each row analysed by 1-way ANOVA. Numbers within a row superscripted by the same letter not significantly different (p = .05) as determined by Scheffé multiple contrasts.

[2] Except where noted, data is for only roots that extended out from the planting core.

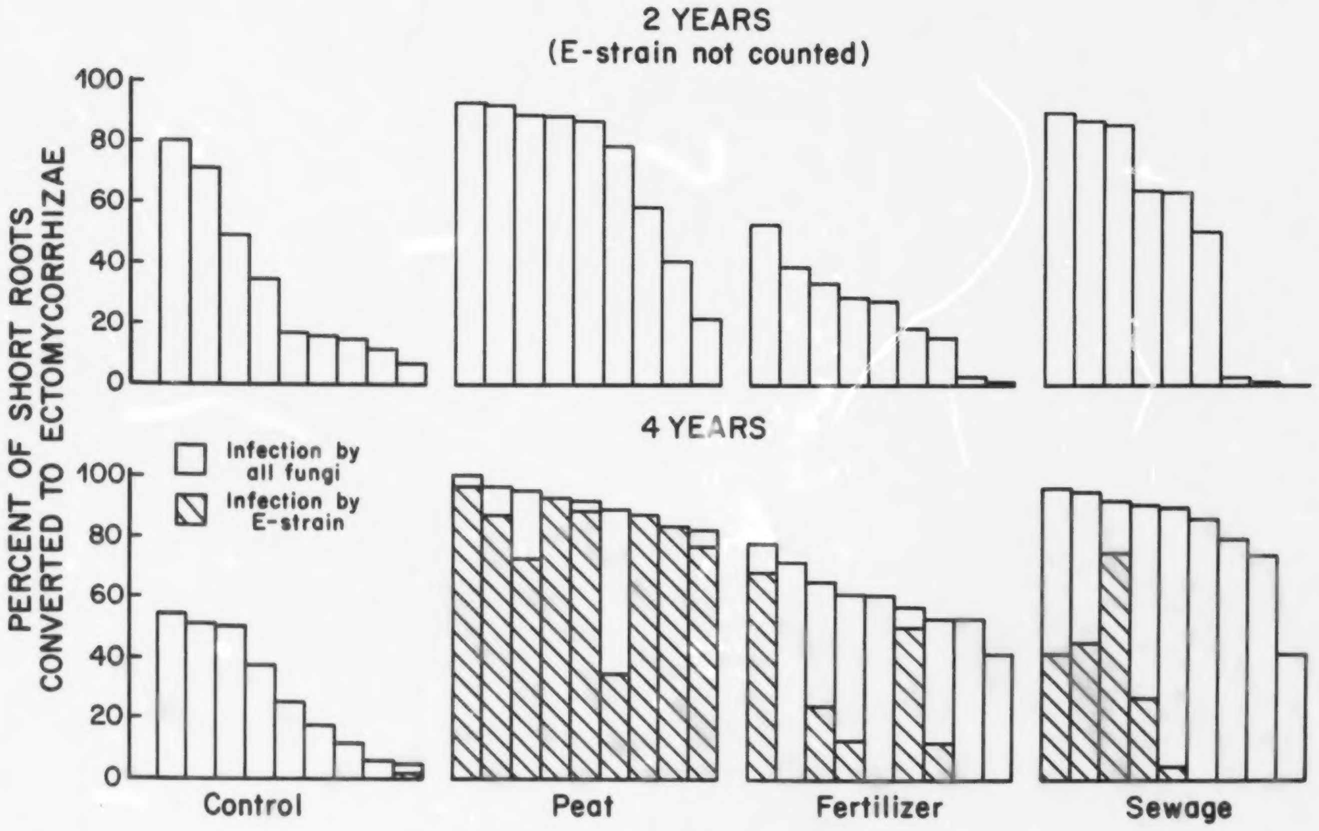

Figure 2. Percent ectomycorrhizal infection of individual jack pine seedlings 2 and 4 years after planting in tailings sand amended with either peat, fertilizer or sewage or left unamended. Indicated is infection by all ectomycorrhizal fungi and E-strain fungi. Each bar represents an individual seedling (nine replicates per treatment).

but the differences were nonsignificant (p = 0.05). Few changes appeared to occur in the long lateral root portions of the root systems between the second and fourth year (compare with data in Table 17). The soil volume sampled each year was approximately equal but it did not include the entire root system as the sample included only the top 20 cm of spoil. Within this volume the amount of roots remained relatively unchanged although there was a considerable increase in the shoot weights (Table 11) except in the control. However, the number of root tips per seedling in the sewage amendment did not appear to increase. This, coupled with an increase in ectomycorrhizal infection (Table 15), suggests that there may have been a considerable increase in the amount of extramatrical mycelium which would effectively increase the absorbing surface of the root system without increases in lateral root length.

Amendment and temporal effects of nonmycorrhizal root parameters were minimal. The amount of short root initiation was slightly greater in the improverished control than in the other three treatments. An increase in lateral root diameter between 2 and 4 years is suggested by the decrease in the length/weight ratio (Tables 16 and 17).

2.4.3.2 Ectomycorrhizal fungi in the planting plug. Preplanting observations on jack pine indicated that most seedlings were heavily infected with ectomycorrhizal fungi. Thelephora terrestris was fruiting on many containers and a single specimen of Inocybe sp. was observed. Thelephora terrestris was cultured from a fruit body and this culture was used for comparison with ectomycorrhiza isolates. Inocybe sp. failed to grow in culture. At the end of the second growing season, T. terrestris was the most common symbiont isolated from roots in the planting plug (Table 18). Suillus was present in plugs from all amendments except the sewage.

Table 17. Characteristics of the root systems of jack pine after four growing seasons. Plants grown in oil sand tailings amended with either peat, fertilizer or sewage sludge, or left unamended.[1]

Root Characteristic[2]	Amendment			
	Control	Peat	Fertilizer	Sewage
No. short roots/ seedling	1002^{a}	2248^{ab}	2477^{b}	9678^{c}
No. root tips/ seedling	1347^{a}	2772^{ab}	3611^{b}	14810^{c}
Lateral root length/ seedling	1.8^{a}	5.5^{b}	5.8^{b}	24.6^{c}
Root weight in soil/ seedling (g)	.27^{a}	.96^{b}	.78^{b}	3.58^{c}
No. short roots/cm lateral root	5.5^{b}	4.1^{a}	4.3^{a}	3.9^{a}
Root length (cm)/ root weight (mg)	.70^{a}	.59^{a}	.74^{a}	.70^{a}
No. tips/short root	1.37^{a}	1.24^{a}	1.46^{a}	1.56^{a}

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[1] Data within each row analysed by 1-way ANOVA. Numbers within a row superscripted by the same letter not significantly different ($p = .05$) as determined by Scheffe multiple contrasts.

[2] Data is for only roots that extended out from the planting core.

Table 18. Isolation frequency of ectomycorrhizal fungi from jack pine ectomycorrhizae on benomyl-MMN agar. Roots sampled after the second growing season from within the planting plug.

Fungi	Amendment Control	 Peat	 Fertilizer	 Sewage
	Percent isolation frequency			
Thelephora terrestris	30	24	41	27
Suillus sp.	7	9	16	0
Basidiomycete	4	3	0	0
No growth of any fungi	0	30	6	1

2.4.3.3 Ectomycorrhizal fungi in the amended tailings sand. On roots growing out from the plugs, T. terrestris was the dominant symbiont isolated from the control, fertilizer and sewage amendments (Table 19). Other Basidiomycete symbionts were rarely isolated. The large percentage of ectomycorrhizae from the peat-amended tailings sand that remained sterile suggested that the symbiont was not T. terrestris and was unable to grow on the benomyl-MMN medium. Procedures were modified in the fourth year so that Ascomycetes could be isolated. Results after the fourth year using benomyl-MMN were similar to the second year results (Table 20) with T. terrestris being isolated at similar rates in each of the two years. Two additional Basidiomycetes were isolated from ectomycorrhizae in the peat treatment, Amphinema byssoides and Tomentella sp., both of which were also isolated from white spruce (see Section 2.2.3). The large percentage of ectomycorrhizae from all treatments which remained sterile on the benomyl-MMN suggests that the species intolerant to benomyl had become more abundant between the second and fourth years. Plating of ectomycorrhizae on MMN+ demonstrated that the benomyl-sensitive species was the E-strain (Table 20). The mantle of ectomycorrhizae formed by T. terrestris was usually very thin or absent and H_2O_2 treatment was lethal to this species. The dominant nonmycorrhizal fungi that were isolated from surface sterilized mycorrhizae were Cylindrocarpon destructans from the peat treatment and Mycelium radicis atrovirens (MRA) from the other three treatments. A single isolate of the ectomycorrhizal discomycete, Sphaerosporella brunnea (Alb. & Schw. ex Fr.) Svrcek & Kub., was obtained from an ectomycorrhiza in the control treatment.

Direct counts substantiated the culturing results (Table 21). The E-strain was dominant in the peat and frequent in the fertilizer and sewage amended tailings sand. The low estimate of the E-strain in the peat using culturing techniques was due to the small portion of the E-strain population indigenous to the peat that was capable of growth on the MMN medium. Evidence strongly suggests that

Table 19. Isolation frequency of ectomycorrhizal fungi from jack pine ectomycorrhizae using benomyl-MMN agar. Roots extending out from the planting plug sampled after the second growing season.

	Amendment			
Fungi	Control	Peat	Fertilizer	Sewage
	Percent isolation frequency			
Thelephora terrestris	73	5	76	44
Suillus sp.	3	1	2	0
Basidiomycete	3	1	1	.5
No growth of any fungi	2	52	4	2

Table 20. Isolation frequency of fungi from jack pine ectomycorrhizae after four growing seasons using either a medium selective for Basidiomycetes (Benomyl-MMN) or a nonselective medium (MMN+).

	Isolation medium[1]	Amendment			
		Control	Peat	Fertilizer	Sewage
		% isolation frequency			
E-strain	Benomyl-MMN	0	0	0	0
Thelephora terrestris[2]	Benomyl-MMN	62^{b}	.6^{a}	60^{b}	51^{b}
Suillus sp.	Benomyl-MMN	2	0	.5	8
Amphinema byssoides	Benomyl-MMN	0	.5	0	0
Tomentella sp.	Benomyl-MMN	0	3	0	0
No growth of any fungi[3]	Benomyl-MMN	24^{a}	37^{a}	33^{a}	12^{a}
E-strain[3,4]	MMN+	2(.4)^{a}	18(11)^{b}	23(6)^{ab}	23(5)^{ab}
Thelephora terrestris	MMN+	2	0	2	6
Suillus sp.	MMN+	0	1	0	0
Cylindrocarpon sp.	MMN+	.5	44	0	.5
Mycelium radicis atrovirens[3,5]	MMN+	41^{a}	0	49^{a}	29^{a}
No growth of any fungi	MMN+	34(24)^{b}	10(5)^{ab}	9(5)^{ab}	12(3)^{a}

[1] Ectomycorrhizal and other fungi isolated from roots extending from the planting core either after washing and plating on benomyl-MMN or after washing, surface sterilization and plating on MMN+.

[2] Data analysed by Krusal-Wallis test. Numbers superscripted by the same letter not significantly different ($p = .05$) as determined by post hoc, multiple comparisons.

[3] Data analysed by 1-way ANOVA. Numbers superscripted by the same letter not significantly different ($p = .05$) as determined by Scheffé multiple contrasts.

[4] Data ln $(x + 1)$ transformed before analysis. Arithmetic means are followed by geometric means in parenthesis.

[5] Peat not included in analysis as there was no variation in replicates.

Table 21. Abundance of four ectomycorrhizal fungi on jack pine planted in oil sand tailings either amended with peat, fertilizer or sewage sludge, or left unamended. Determinations made after four growing seasons using direct counts of short roots and ectomycorrhizae.

	Amendment							
	Control	Peat	Fertilizer	Sewage	Control	Peat	Fertilizer	Sewage
	% of short roots ectomycorrhizal with:				Number of samples each type occurred in of 9 possible			
E-strain[2]	0.2(0.6)	80(19)	18(25)	21(27)	2	9	5	5
Suillus sp.	1.7	0.9	1.8	6	4	1	5	2
I-type ascomycete	0	1.8	0	0	0	1	0	0
Amphinema byssoides	0	0.5	0	0	0	1	0	0

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[1] Data not analysed due to the large number of samples with zero frequencies.

[2] Mean and standard deviation.

E-strain inoculum was introduced with the peat but the source of the inoculum in the other treatments is unknown.

When the second year assays were made, the E-strain ectomycorrhizae could not be recognized and it thus is not known for certain if the frequency of E-strain mycorrhizae changed between 2 and 4 years. However, the possibility of there being more than one symbiont forming the rather nondescript ectomycorrhizae was investigated after the second growing season. Forty samples of ectomycorrhizae were selected to cover the range of morphology, colour and mantle characteristics; photographed, and either plated directly on benomyl-MMN or surface sterilized prior to plating. Of the 27 types from the control, sewage and fertilizer treatments, 25 yielded cultures of *Thelephora terrestris* and two yielded no symbionts. It is possible that these two types were E-strain ectomycorrhizae. Of the 13 types from the peat, a subtuberculate type yielded cultures identical to *Suillus tomentosus* and the other 12 yielded no symbionts while 86% of the surface sterilized tips remained sterile. It appears that if the E-strain was present in treatments other than the peat at the end of the second year, it was rare.

Based on the 25 ectomycorrhizal samples that yielded cultures of *T. terrestris*, ectomycorrhizae formed by this fungus were monopodial to coralloid with up to 16 tips per short root, glabrous, lacking mycelial strands or rhizomorphs, elements short and robust to long and slender, buff to pale snuff-brown or occasionally snuff-brown. The Hartig net was well developed but the mantle was usually very thin or lacking and there was no intracellular penetration.

2.5 DISCUSSION OF AMENDING OIL SAND TAILINGS

Plants in the amended oil sand tailings became mycorrhizal either by virtue of inoculum present in the planting core, inoculum introduced with the peat and, possibly, by air-borne sources. It is apparent that once a mycorrhizal fungus became established in spoil material, changes in mycorrhizal associations were slow to occur.

Plants that lacked inoculum in planting plugs only slowly became mycorrhizal, presumably by mycelium from neighboring plants. Some jack pine seedlings had very low mycorrhizal infection rates even after 2 years. The consequence of having some plants with very low infection rates after 2 years in the field was a significant decrease in variability with time. Thus a portion of the jack pine seedlings were unable to benefit from mycorrhizal associations for several years when container-grown seedlings were used in inoculum poor spoil.

Amendation affected the rates of colonization by mycorrhizal fungi, in the case of the peat, by increasing the inoculum density and thus decreasing the variability. Fertilizer had no effect on rates of ectomycorrhizal development for the first 2 years but then caused an increase in ectomycorrhizal infection rate compared to the control due to better seedling growth. Sewage both greatly enhanced plant growth and ectomycorrhizal development of jack pine while depressing ectomycorrhizal formation and having a smaller effect on growth of bearberry.

Thelephora terrestris + jack pine ectomycorrhizae were very difficult to identify even when using high magnification techniques. It was necessary to use culturing techniques and comparison with known fruit body isolates to identify and quantify T. terrestris ectomycorrhizae. Frequency of recovery of T. terrestris from surface sterilized ectomycorrhizae was very low and this was probably due at least in part to the very thin mantle. The formation of a thin mantle or its complete absence on jack pine has been previously noted by Marx and Bryan (1970) and Hacskaylo (1965). Thus it appears that mantle development is normally limited in jack pine + T. terrestris ectomycorrhizae. Ectomycorrhizae formed by T. terrestris have been described as having hyphal strands (Hacskaylo, 1965; Marx et al., 1970; Thomas and Jackson, 1979) but no strands were associated with jack pine + T. terrestris mycorrhizae in this study. This suggests a taxonomic difference between Alberta and southern U.S. Thelephora's. Thelephora terrestris is also noted for its ability to fruit on and around young seedlings in nurseries (Corner, 1968). *Thelephora terrestris* was not observed to fruit in the amended sands until the fourth year and then only in the sewage treatment. In southern pine nurseries and on plots amended with sewage, *T. terrestris* may fruit prolifically during the first growing season (Berry and Marx, 1976; Marx et al., 1970). It appeared that the *T. terrestris* strain introduced with the seedlings required large plants prior to fruting, perhaps due to plant induced environmental modifications as it fruited on the containers prior to outplanting.

Although the data from the second year were not detailed with respect to specific symbionts, it appeared that the E-strain was stable within the peat and increased in abundance in the other treatments. The E-strain fungus is considered to be a species of low competitive abilities and thus is rapidly replaced by other fungi (Mikola, 1965). In particular, Thomas and Jackson (1979) stated that in spruce nurseries, the E-strain was replaced by *Thelephora terrestris*. In oil sand tailings, the opposite appeared to take place with jack pine. In addition, *T. terrestris* was largely able to colonize roots in peat amended tailings sand in the presence of the E-strain. From the data gathered, it appeared that the E-strain persisted longer in amended sands than *T. terrestris*. Although symbionts typical of mature forests (*Suillus* sp. and *Cenococcum geophilum*) were present, they did not appear to increase in abundance and remained minor components of the ectomycorrhizal populations.

3. CHLAMYDOSPORE POPULATIONS OF THE E-STRAIN SYMBIONT IN AMENDED COAL MINE SPOIL.

3.1 INTRODUCTION

The E-strain fungus, which was the dominant symbiont associated with white spruce (see Section 2.2.3), is unusual in that it produces large soil-borne chlamydospores (Danielson, 1982). It is likely that these chlamydospores serve as resistant resting propagules and are important sources of inoculum. They may function to infect roots of newly introduced plants or to re-infect roots of established plants following periods of severe stress that are lethal to mycelium. It was thus of interest to know if the type of amendment affected chlamydospore populations and whether or not the E-strain symbiont is more or less beneficial than other symbionts that occur in the spoils. Chlamydospore populations in the subalpine coal mine spoil were quantified in the spring of 1980:

1) to determine the effects of peat, sewage sludge and fertilizer on the production of chlamydospores by the E-strain;
2) to determine if any chlamydospores of VA mycorrhizal fungi were present in the spoil with a nonhost species; and
3) to compare amendment effects on chlamydospore populations of two dissimilar mycorrhizal systems, i.e. E-strain + spruce and VA + slender wheatgrass (see Zak et al., 1984).

3.2 MATERIALS AND METHODS

Three randomly chosen soil samples per plot (three replicate plots for each type of spoil amendment) were taken prior to any new plant growth in spring 1980. The samples were taken to a depth of 10 cm and located 3 cm north of each randomly chosen seedling. The soil fraction larger than 4 mm was discarded, the roots were removed for separate examination and the remaining soil was stored at 5°C until it could be processed.

Twenty-five grams of mineral soil (wet weight) or 10 g of peat of each sample were added to about 350 mL water and mixed with a magnetic stirrer for 3 min. The soil suspension was wet sieved through a series of 500 m, 250 m, 125 m and 53 m sieves. The material on the 53 m and 125 m was washed into centrifuge tubes and brought up to volume with water. The tubes were centrifuged at 2000 RPM for 10 min and after standing for 15 min, the liquid decanted onto coarse filter paper in a Millipore filter holder under a vacuum. Additional filters were used if a large amount of suspended material was present. The material in the tubes was then resuspended in a 2M sucrose solution and centrifuged again at 2000 RPM for 10 min and filtered as above. The filters were scanned at 50X and all E-strain chlamydospores and all VA-type spores picked off the filters. The VA-type spores were mounted on slides in lactophenol for examination and the E-strain spores were mounted in Shear's mounting fluid or lactophenol. This method was tested by adding a known quantity of spores to peat and successively extracting more than 50% of them (Appendix Tables 4 and 5).

In order to determine if the E-strain fungus was present in each sample, the mycelium and mycorrhizae were examined. Each filter from the 125 μm-sucrose extraction was examined for E-strain hyphae. The criteria used to identify the E-strain hyphae were (1) the size range of 4-6 μm diameter, (2) the presence of ornaments in the form of small blisters, (3) simple septa with associated Woronin bodies and plugs, (4) brown pigmentation evident at 30X magnification, and (5) infrequent branching. The mycorrhizae were closely examined for diagnostic characteristics in whole mounts using a 40X oil immersion objective. The roots found in each sample were dried and weighed to determine if the number of chlamydospores was related to the amount of roots present.

3.3 RESULTS

E-strain ectomycorrhizae and hyphae were present in all treatments (Table 22). The quantity of roots in all treatments was similar and thus did not preclude the production of chlamydospores if conditions were otherwise favorable. There was no apparent correlation between the amount of roots and the number of chlamydospores.

Very few chlamydospores were produced in the peat and sewage amended spoil while substantial numbers were found in the control and fertilizer amended spoils. These differences were not due to differences in seedling sizes as these were similar in all treatmets (see Table 3). The greatest number of spores in any one replicate was 739/100 g dry soil in one control sample.

Table 22. Numbers of E-strain chlamydospores in amended coal mine spoil, presence of E-strain hyphae and mycorrhizae and VA chlamydospores after 3 years growth of white spruce.

Amendment	Amount of root in sample (mg)	No. of samples (of 9 possible) with E-strain:		E-strain spores per 100 g dry soil	VA-type chlamydospores per 100 g dry soil[2]
		Mycorrhizae	Hyphae		
Control	20	5	9	174^{a}	28^{a}
Peat	21	8	8	0	1118^{b}
Fertilizer	32	9	6	177^{a}	28^{a}
Sewage	52	8	9	3	36^{a}

[1] A Chi-square goodness-of-fit test was used to determine if amendment affected the number of samples with spores. Control and fertilizer had significantly greater numbers of samples with spores. They were then tested by a t-test to determine if the actual numbers of spores produced were different.

[2] Data analysed by Kruskal-Wallis test. The difference in means was determined by post-hoc multiple comparisons. Numbers within the same column superscripted by the same letter not significantly different (p = .05).

VA-type chlamydospores were rare in all treatments except the peat. Very few of these spores appeared to be viable and were probably introduced with the peat.

Water was ineffective in floating out the chlamydospores of the E-strain (Appendix Tables 4 and 5). A much higher percentage of VA-type spores were extracted with water, however, almost all of these were nonviable.

3.4 DISCUSSION

Amendation had a strong influence on the production of E-strain chlamydospores. The suppression of spore production in the sewage amended spoil was apparently not due to plant size, which was similar in all treatments, nor to the absence of E-strain mycorrhizae. At the end of the fourth year, the E-strain infected 47% of the short roots in the sewage amended spoil and 80 and 94% of the roots in the control and fertilizer amended spoil, respectively. A similar suppression of Glomus aggregatus spore production in the sewage treatment occurred in the slender wheatgrass plots (Zak et al., 1984). The lack of spore production and the gradual replacement of the E-strain on the roots by Amphinema byssoides (see p. 26) may indicate that conditions in sewage treated spoil was somewhat unfavorable for the E-strain symbiont.

The lack of E-strain chlamydospores in the peat was not an artifact of the extraction procedure (see Appendix Tables 4 and 5) but is most likely due to the presence of another taxon of the E-strain. E-strain isolates for the peat differed significantly from those in the other treatments and they did not produce chlamydospores in culture (Danielson, 1982). The absence of chlamydospores in the peat suggests that the E-strain inoculum from the spoil has been unsuccessful in competing with the taxon that was introduced with the peat.

The procedure used here for extracting chlamydospores should be modified when the substrate is peat or forest floor material. The water extractant and the 125 μm sieve washings can be eliminated. It is recommended that settling times of 15 and about

75 min be used and then a 15 min settling time after resuspension and centrifugation.

4. ECTOMYCCORHIZAL INOCULATION IN THE CANMORE PEAT USED AS AN AMENDMENT.

4.1 INTRODUCTION

Data for mycorrhizal infection of jack pine strongly indicated that the peat was a source of the inoculum of the E-strain (see Section 2.4.3.3). However, no initial evaluation of the peat was made for ectomycorrhizal inoculum. In the subalpine spoil, the E-strain was present in all treatments and could have been introduced with the spoil or in the root plugs as well as with the peat. However, no chlamydospores were recovered by wet sieving the peat around the spruce seedlings (see Section 3) or the spoil in the absence of spruce (see Zak et al., 1984). Thus the source of the E-strain inoculum was still questionable. Information on the source of the inoculum will increase our knowledge of the ecology of this symbiont and may permit predictions on mycorrhizal associations in other revegetation studies. It was felt that it would be possible to determine the potential inoculum source of the E-strain by sampling the site at Canmore in June 1980 where the peat was obtained in 1977. The objectives for resampling the Canmore peat were:

1) to determine if the E-strain symbiont was present in the peat deposit;
2) to determine if chlamydospores of the E-strain were present in the peat; and
3) to determine what other symbionts compatible with jack pine were indigenous to the peat.

4.2 MATERIALS AND METHODS

Peat was collected from the Canmore site in June, 1980. The area was forested with mature white spruce with a shrub undergrowth. Three profiles (soil pits) were sampled around the perimeter of the excavated peat site. The face of each profile was cleaned and samples taken at the 20-30, 50-60, and 80-90 cm depths from each of three profiles. When the peat was originally harvested, the surface layer was stripped off and discarded. The top sample (20-30 cm) was within the rooting zone of the surrounding white spruce and the peat was fairly soft and fibrous. The middle depth (50-60 cm) contained few roots and the peat was much more amorphous. The lowest depth (80-90 cm) was at the bottom of the profile just above the clay bottom of the old bog. The material at the bottom was hard and was removed in layers. A few roots reached the bottom of each profile. An area of approximately 50 x 15 cm was removed from each depth. The peat was stored at 5^0C until it could be broekn up by hand and thoroughly mixed.

For detection of E-strain chlamdospores, 10 g samples were wet-sieved as described in the E-strain chlamydospore section (Section 3.2). To determine if viable propagules of the E-strain and other symbionts were present, a baiting technique was used. Jack pine was used rather than white spruce due to the more rapid growth of jack pine and the more distinctive E-strain mycorrhizae formed with jack pine than with spruce.

Peat from each sample was put into two 15.5 cm diameter pots and the surface covered with a layer of granite grit. One sample of the 20-30 cm depth from each pit was autoclaved and pots prepared in the same manner to serve as indicators of air-borne contamination. All pots were planted with three surface sterilized (30% H_2O_2 for 30 min), pregerminated jack pine seeds and one-half of the pots were watered twice weekly with a solution of 75 $mg.L^{-1}$ of 20:20:20 fertilizer containing trace elements and iron (Plant Prod). To the other half (i.e. one pot of each sample), water only was added. The plants were harvested when they were 18 weeks old, the tops dried and the roots cleaned and evaluated for mycorrhizal infection.

The degree of infection was estimated by examining the short roots with a dissecting microscope and checking for infection by observing wholemounts at 500X magnification. Each morphological type was estimated separately and isolation procedures were used to confirm differences among types. For isolations, the mycorrhizae were washed under cold, running water for 1 to 2 h, either plated directly or surface sterilized with 30% H_2O_2 for 15 sec and plated on MMN+ and benomyl-MMN agar. Chemical analysis of the peat was performed according to McKeague (1976).

4.3 RESULTS

There was no sighnificant effect of depth or profile location on soil pH or levels of NO_3-N (Table 23). Ammonium-N did not vary with depth but was significantly higher in profile 3 than in the other two sites. Due to depth-site interactions, the P data could not be analysed but it is obvious that P concentrations were very low in all samples except the one from the bottom of profile 3. This sample was unusual in virtually all factors examined and contributed a major portion of the variation. Autoclaving resulted in a large release of NH_4-N, no change in NO_3-N and a nonsignificant increase in the level of extractable P.

The jack pine seedlings grew very poorly in all the unfertilized samples except in the surface sample of profile 3 (Table 24). All seedlings in the unfertilized series, except those in the 20-30 cm depth of profile 3, showed P deficiency symptoms. Fertilization strongly stimulated growth of jack pine in all samples except those from the 80-90 cm depth of profile 3. This sample had a visible slimy growth of bacteria at the time of sampling and the pot had an anaerobic odor when sampled. The amount of stimulation by fertilization was quite variable and was not consistent with depth. Seedlings in three pots of the fertilized peat showed apparent Fe deficiency despite the fact that the fertilizer contained Fe. The apparent Fe deficiencies could not be related to the ectomycorrhizal status of the roots. Autoclaving the peat also stimulated seedling growth by releasing nutrients. Addition of fertilizer to the autoclaved peat had no apparent effect on growth.

No ectomycorrhizae were formed in the autoclaved peat, indicating that most or all infections in the nonsterilized pots were the result of indigenous inoculum. Ectomycorrhizal inoculum

Table 23. Chemical characteristics of the Canmore peat.[1]

	Depth (cm)	pH	NO_3^--N ($\mu g.g^{-1}$)	NH_4^+-N ($\mu g.g^{-1}$)	P ($\mu g.g^{-1}$)
Profile 1	20-30	5.8	242	14.5	2.5
	50-60	5.8	630	15.5	1.7
	80-90	6.3	714	12.5	1.2
Profile 2	20-30	6.4	104	11.5	1.5
	50-60	6.0	270	7.0	1.5
	80-90	6.2	396	10.5	2.5
Profile 3	20-30	5.8	468	20.0	3.0
	50-60	5.7	374	38.0	1.2
	80-90	6.0	44	26.0	35.0
Autoclaved					
Profile 1	20-30	-	248	512	7.5
Profile 2	20-30	-	105	407	2.5
Profile 3	20-30	-	396	462	12.5

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[1] Data for nonautoclaved sampled analyses by 2-way ANOVA, without interactions after testing for interactions using Tukey test for additivity. Effect of autoclaving tested by paired t-test, differences tested at $p = .05$.

Table 24. Growth of jack pine on Canmore peat from three pits and three depths with and without fertilization.[1]

	Pit 1		Pit 2		Pit 3	
Depth (cm)	Fert	Non Fert	Fert	Non Fert	Fert	Non Fert
			Shoot weight (mg)			
20-30	107^{bcd}	50^{abc}	117^{cd}	40^{ab}	893^{g}	160^{de}
50-60	206^{def}	29^{a}	344^{efg}	41^{ab}	677^{fg}	53^{abc}
			Shoot weight (mg)			
80-90	227^{b}	43^{a}	394^{b}	38^{a}	36^{a}	32^{a}
			Root weight (mg)			
20-30	24^{a}	29^{a}	27^{a}	24^{a}	209^{b}	60^{ab}
50-60	48^{ab}	20^{a}	97^{ab}	30^{a}	171^{b}	23^{a}
			Root weight (mg)			
80-90	58^{a}	24^{a}	116^{c}	22^{a}	20^{a}	22^{a}
			Autoclaved - Shoot weight (mg)			
20-30	160	160	100	78	660	417
			Autoclaved - Root weight (mg)			
20-30	30	76	41	41	141	194

---

[1] Data were separated into 2 groups based on depth. The shoot and root weight data for 20-30 cm and 50-60 cm group was analysed by 3-way ANOVA. The 80-90 cm group was analysed by 2-way ANOVA. Numbers within a group superscripted by the same letters not significantly different ($p = .05$) as determined by Scheffe multiple contrasts where F-test significant.

[2] Autoclaved data not analysed.

was present throughout the peat profile (Table 25). The only sample in which there was no infection was the one from the 80-90 cm depth of profile 3. It was estimated that at least 13 species of symbionts infected jack pine. There did not appear to be qualitative differences with depth except possibly in pit 3 where the E-strain, a form designated 'glabrous-dark buff' and *Amphinema byssoides* were restricted to the 20-30 cm depth. *Amphinema byssoides* occurred in the 20-30 cm depth in all three profiles but did not occur at the 80-90 cm depth in any of the three profiles.

Ectomycorrhizal development was strongly stimulated by application of mineral fertilizer. Considering all depths, infection was 48 and 87% in nonfertilized and fertilized pots, respectively. The most abundant symbiont was an Ascomycete designated as the I-type. This symbiont (or perhaps two or more similar species) formed greater than 78% of the ectomycorrhizae in seven of the nine fertilized profile-depth combinations. This symbiont formed ectomycorrhizae with jack pine which were simple or dichotomous, pale fulvous, inflated, glabrous or with hyaline cystidia which arose from the outermost cells of the mantle. The cystidia were of a setoid type, up to 100 μm long, thin-walled, septate with acute tips. Woronin bodies were associated with the septa of the setae. The mantle was a textura epidermoidea, perfectly smooth except for the setae with no extramatrical mycelium or mycelial strands. This form conforms to the I subtype of Dominik (1962).

A second cystidial type was less frequently found and was assigned to the genus *Tomentella*. The ectomycorrhizae were simple to dichotomous, snuff-brown to umber and glabrous except for the presence of light-brown setae. The setae were 2.5 μm at the base, clamped where attached to the mantle, walls slightly thickened, pale-brown, about 200 μm long with acute tips. The mantle was a textura epidermodea with cells up to 6 μm diameter. The culture was moderately fast growing, pale-brown to hyaline on MMN, dark-brown with a black reverse on PDA. The hyphae were clamped, 4 μm diameter and verruculose. ON PDA brown, spinulose crystals 6-8 μm in diameter were produced in the agar or brown pigments occurred within

Table 25. Fungi forming ectomycorrhizae with jack pine planted in peat from under a white spruce stand under greenhouse conditions.

	External features*	Hyphal pigments**	Sampling Depth(cm)			Number of seedlings colonized (of 18)	Percent Infection of colonized seedlings
			20-30	50-60	80-90		
			Percent Infection+				
Ascomycetes							
I-type	Cystidia	-	47	47	33	9	84
E-strain	EMM	+	16	0	0	1	99
Cenococcum geophilum	EMM	+	<1	0	0	1	<1
Basidiomycetes							
Tomentella sp.	Cystidia	+	<1	11	<1	7	10
Amphinema byssoides	EMM+R	-	3	1	0	5	2
Rhizopogon-like sp.1	EMM+C+R	-	0	0	1	1	<1
Rhizopogon-like sp.2	EMM+C+R	-	0	0	1	1	1
Unknown 1	EMM	+	16	11	0	2	82
Unknown 2	EMM	+	1	0	0	1	9
Unknown 3	Glabrous	-	13	0	0	1	76
Unknown 4	EMM	-	0	1	0	1	5
Unknown affinity		-	<1	<1	<1	4	1

* EMM = extramatrical mycelium, C = crystals on the mycelium, and R= rhizomorphs or mycelial strands.

** Individual hyphae with brown pigments.

+ Represents the mean of six plants per sampling depth.

the hyphae. These brown materials turned green with the application of 3% KOH or 10% NaOH and dissolved.

Two other species of Basidiomycetes with brown pigmented hyphae were observed and isolated. One was very slow-growing and the other was similar to Tomentella sp. but lacked the KOH-reactive pigments. It formed ectomycorrhizae only with nonfertilized seedlings. Fertilized seedlings in the same peat samples were dominated by I-type ectomycorrhizae.

The E-strain symbiont formed ectomycorrhizae in only one of the nine samples. The infection was typical for this species, having a thin mantle and intracellular hyphae. Although mycorrhizal tips plated on MMN produced the typical straight, ornamented hyphae, only one isolate grew enough to be subcultured. This isolate was identical to isolates from the peat amended spoils and did not produce chlamydospores in culture. No E-strain chlamydospores were recovered by wet sieving of the nine samples. Other symbionts in the Canmore peat included Cenococcum geophilum, which was very rare, Amphinema byssoides, which occurred at low densities, several unknowns and two isolates which were similar in appearance to Rhizopogon species.

The use of isolation procedures was useful in symbiont identification as 10 of the 13 fungi were successfully isolated. Root washing plus direct plating of mycorrhizae on MMN+ or benomyl-MMN was the most successful isolation technique with 64% recovery from 250 mycorrhizae. When the mycorrhizae were also surface sterilized, the recovery was only 19% from 165 tips.

4.4 DISCUSSION

E-strain ectendomycorrhizae were formed in one sample of the Canmore peat. Isolation attempts and wet sieving for chlamydospores indicate that the taxon in the Canmore peat is distinct from that in the subalpine spoil and that it does not produce chlamydospores either in culture or in the peat substrate. However, it does appear that the E-strain taxon from the peat produces resistant propagules. A sample of the original peat amendment which had been stored outside in a small pile for 3 years was planted with jack pine. After 13 weeks growth, all the seedlings were infected with the E-strain. No chlamdospores could be recovered by wet sieving from this stored peat. It appears that resistant spores are produced by E-strain fungi in addition to large chlamydospores.

Although no pines were on the sampling site there was a large number of nonhost-specific symbionts. It can be assumed that most or all the symbionts recorded here also formed mycorrhizae with white spruce. It would appear that the use of peat formed under spruce stands would provide a wide spectrum of symbionts compatible with pines. In the experimental soil tanks the only symbionts apparently introduced successfully with the Canmore peat were the E-strain and Tomentella.

The identity of the I-type is unknown but its Ascomycete affinity is clear. It is unusual that nearly all of the mycorrhizae formed on the fertilized jack pine had Ascomycete symbionts. The frequency of Ascomycete symbionts of the native white spruce in the field was not determined. Future studies on ectomycorrhizae in organic spoils should examine the relative importance of Ascomycetes in more detail.

The ectomycorrhizae formed with jack pine grown in the Canmore peat had two distinct morphological characteristics that were uncommon or absent in native jack pine mycorrhizae in sandy, well-drained soils (i.e. Mildred Lake) (see Section 5.2). Firstly, four tentative taxa of Basidiomycetes plus the E-strain found on jack pine grown in Canmore peat all produced pigmented hyphae, whereas in the jack pine from the field site, symbionts with brown pigmented hyphae were very rare. It is likely that all four of the Basidiomycetes from the Canmore peat are in the Aphyllophorales and it would appear that the nonhost--specific symbionts in the peat are not agarics. Fruit bodies of Gasteromycetes, Corticium-like fungi and Ascomycetes should be collected and cultured to try to identify these symbionts.

The second morphological characteristic of interest is that mycelial strands, rhizomorphs and extramatrical hyphae were rarely associated with the ectomycorrhizae of plants grown in the Canmore peat. The only types which had mycelial strands were the Rhizopogon-like and Amphinema byssoides mycorrhizae, all of which were uncommon. Species with a loose network of extramatrical mycelium included the E-strain, Brown Basidio, Cenococcum geophilium and Triangular branch types. The low incidence of ectomycorrhizae with mycelial strands suggests that these structures are of less importance in wet organic soils than those subject to periodic water stress. In jack pine growing at Mildred Lake, glabrous ectomycorrhizae were uncommon and well-developed rhizomorphs were common (See Section 5.2). In the peat, where water conductance and ion diffusion rates would be expected to be high, the glabrous or cystidial type may be equally effective in water and nutrient uptake as rhizomorphic species. Nonglabrous species would also have to expend carbon and energy on the production of rhizomorphs or extramatrical mycelium.

The cystidial morphological type would appear to be intermediate between rhizomorphic and glabrous types. The cystidia would effectively increase the surface area and amount of soil exploited but the amount of extension would be genetically limited unlike the unlimited potential growth of mycelial strands. However, the actual production of cystidia is apparently environmentally controlled as in the Canmore peat the I-type mycorrhizae exhibited a great range in the quantity of cystidia produced, i.e. glabrous to bristling with cystidia. Thus during dry periods, the fungus may expend energy to produce cystidia with a resultant increase in the capacity for water uptake and be able to conserve energy during wet periods by not producing cystidia. It therefore follows that the symbionts native to peat may not be as effective on reclaimed land surfaces as would be rhizomorphic species.

To obtain the maximum benefit from ectomycorrhizal infection in reclamation efforts, it still may be desirable to establish symbionts which possess morphological adaptations for extracting water and nutrients in dry, low conductance soils. For example, the mycelium of Cenococcum geophilium, a species reputed to be effective in droughty situations (Trappe, 1977), can extend over 2 m from the roots (Fogel, 1980) thus vastly increasing the volume of soil exploited. Duddridge et al. (1980) have shown that water can be transported through rhizomorphs of *Suillus bovinus* for distances more than 0.5 m and be utilized by the host. The use of peat as a source of inoculum may introduce nonagaric, nonrhizomorphic symbionts ill-adapted to the variable moisture conditions and high soil temperatures that are likely to be found on reclaimed mine spoils.

5. FIELD STUDIES

5.1 ECTOMYCORRHIZAL FUNGI ASSOCIATED WITH SPRUCE AT THE SUBALPINE COAL MINE SITE.

5.1.1 Introduction

The spoil that was used in the tank study was from a coal mine at Luscar, Alberta. It was thus of interest to obtain some information in the ectomycorrhizal associations that occurred in both undisturbed and disturbed sites for comparison with the associations that developed in the tank study. The objective of this brief study was to determine the identity of ectomycorrhizal fungi occurring in the subalpine site.

5.1.2 Materials and Methods

The spruce-fir site has been described briefly in Section 2.1.1 and in more detail by Visser et al. (1984). White spruce naturally regenerating in adjacent areas, an abandoned roadbed and a roadcut, were sampled to determine the symbionts occurring in disturbed areas. Five samples of the forest floor in the mature stand, 15 x 15 x 5 cm, were taken in June 1978. Five spruce seedlings were dug up from the roadbed in June and five more removed from a roadcut in another location in September. The roots were washed free of litter and soil and examined. All active ectomycorrhizae (Appendix 1) in the forest samples were counted and all ectomycorrhizae formed by Cenococcum geophilium were counted in the June samples. After direct examinations, the roots were cut into 2-3 cm segments and 10 segments from each sample were randomly selected and the first five ectomycorrhizae on one end surface sterilized in H_2O_2 and plated on benomyl-MMN. Twenty ectomycorrhizae from each sample were also plated without being surface sterilized but Mortierella sp. was so abundant that no symbionts could be isolated.

At the time the isolation sampling was done, criteria for the identification of the E-strain were not developed and it was not known if this fungus was indigenous to the subalpine site. In that the E-strain produces large soil-borne chlamydospores that can be recovered by wet sieving, the area was resampled in June, 1981 and the soils sieved. The undisturbed area was sampled by removing 15 cm square cores every 4 m along a transect. The forest floor (LFH layers), which was about 5 cm deep, was sampled separately from the clayey mineral soil which was sampled to a depth of 5-10 cm. Five volunteer white spruce seedlings growing on a roadcut were dug up as well as five seedlings that had been transplanted from a roadcut 2 years previously and planted on reclaimed spoil. These 20 samples were wet sieved as described previously (see Section 3) and examined for E-strain chlamydospores.

5.1.3 Results and Discussion

Ectomycorrhizae formed by Cenococcum geophilum were the only ones that were distinctive enough to be quantified by direct counts. Cenococcum geophilum was not present on any of the seedlings growing in the abandoned roadway (mean dry root weight: 238 ± 120 mg, x ± SD/sample). In the mature stand, 14.5 ± 9.5% of the ectomycorrhizae were formed by C. geophilum. The samples, about 1L in volume, contained 746 ± 505 ectomycorrhizae on 458 ± 190 mg of roots. All ectomycorrhizae in the abandoned road samples were indistinguishable from those formed by Amphinema byssoides. The most common symbiont recovered from the surface sterilized ectomycorrhizae was A. byssoides (Table 26). It formed slow growing, cream coloured

Table 26. Fungi isolated on benomyl-MMN from surface sterilized white spruce ectomycorrhizae from a mature stand and from seedlings in cutlines at the subalpine coal mine site.

Taxa	Roadcut (June)	Mature Stand (June)	Roadcut (Sept.)
	% isolation frequency[1]		
Amphinema byssoides	41	18	16
Lactarius-like	3	0	17
Basidiomycete	5	0	.4
Sterile hyaline	12	15	34
Mortierella sp.	5	5	1
Mucor sp.	1	.4	0
Tolypocladium sp.	1	0	0
Unknown	1	4	0
Yeast	0	0	2
No growth of any fungi	31	60	30

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[1] Fifty ectomycorrhizae plated for each sample site.

colonies that turned pale to bright yellow when a drop of 2% KOH, 10% KOH or concentrated NH_4OH was applied. The colonies also reacted with phenol aniline (Singer, 1975) to turn livid vinaceous within 2 min. Colonies did not react to H_2SO_4, formalin, phenol, $FeCl_3$ or sulfoformal. The hyphae were 2-3 m diameter, clamped with keyhole- type clamps and smooth or lightly encrusted.

Lactarius-like fungi were the only other symbionts isolated. They produced slow-growing colonies lacking aerial hyphae except for erect fascicles in the center. The hyphae were hyaline, smooth and with simple septa. *Piloderma croceum* Erikss. & Hjortst. was also present in one sample from the mature forest as evidenced by the golden yellow mycelium (Mikola, 1961) in the forest floor but it was not isolated.

Chlamydospores were found in only one sample, a seedling sample from the cutline. Thirty chlamydospores were recovered from 25 g of soil. This spore density was similar to that found in the tanks (see Table 22). This observation demonstrates that E-strain inoculum is present in disturbed subalpine systems and suggests that the E-strain in the spoil in the tanks may have originated with the spoil itself.

5.2 ECTOMYCORRHIZAL FUNGI OCCURRING IN A JACK PINE-LICHEN WOODLAND LOCATED IN NORTHEASTERN ALBERTA.

5.2.1 Introduction

The ectomycorrhizal inoculum for jack pine in the tank study originated from either the peat, air-borne spores or was introduced with the planting stock. It is thus unlikely that the ectomycorrhizal associations that developed in the tank study would be similar to those developing under natural conditions. In order to determine the degree of similarity, ectomycorrhizal associations were studied in a native jack pine-lichen woodland near Fort McMurray, Alberta where oil sands are currently being mined. The data obtained from this study should serve to determine the species diversity of ectomycorrhizal fungi, the abundance of morphologically distinctive ectomycorrhizae and allow comparisons between disturbed and undisturbed jack pine stands. In addition, the collection of fruit bodies will provide valuable cultures which can be used in future inoculation programs and for comparison with unknown ectomycorrhizal isolates.

5.2.2 Materials and Methods

The study site was located adjacent to the Alberta Environment Mildred Lake Research Facility about 40 km north of Fort McMurray, Alberta (57°5'N, 111°45'W). The jack pine stand was about 40 years old with little ground vegetation other than lichens (primarily Cladina mitis). Adjacent to this stand was an area cleared several years previously of vegetation and regenerating to jack pine and bearberry. This area (cutline) was used as an example of a disturbed site for comparison with the jack pine-lichen woodland.

The identify of ectomycorrhizal fungi was determined by two methods:

1) Fruit bodies of all suspected symbionts in the vicinity of the plot were collected on 22 September 1977, 29 September 1978, 20 June 1979, 20 September 1979, 2 July 1980 and 16 September 1980. Collections were made from the mature stand and along the adjacent cutline. These collections provided qualitative information on a portion of the ectomycorrhizal symbionts but no information on the abundance of any symbiont-host combination.

2) Fifteen cores, 10 cm deep and 5.7 cm in diameter, were taken at 1.5 m intervals on a transect across the stand on 20 June and 20 September 1979. The September cores were taken 15 cm from the June samples. Ten cores, 10 cm diameter and 15 cm deep, were also taken adjacent to jack pine seedlings in the cutline. The roots in all the cores were washed free of soil and all active ectomycorrhizal tips (not short roots) were counted.

Specific ectomycorrhizae were identified on the basis of similarity to ectomycorrhizae previously synthesized in pure culture (R.M. Danielson, unpublished data). Briefly, jack pine + Tricholoma flavovirens ectomycorrhizae were characterized by (1) elements monpodial or irregularly branched 1 to 3 times, loosely arranged, (2) colour pale livid vinaceous to livid vinaceous, hyaline if hyphae sparce, (3) hyphal strands abundant, concolourous with the ectomycorrhizae, branching into fine strands.

Jack pine + Lactarius paradoxus ectomycorrhizae were (1) simple to coralloid and robust, (2) mantle very smooth, appearing as a translucent halo in profile, (3) rhizomorphs fused to lateral roots, (4) ectomycorrhizae and rhizomorphs cream to brown and tinted grey-green at least in part, (5) in plan view, mantle a textura epidermoidea. Other members of the subgenus Lactarius probably form very similar ectomycorrhizae with green pigmentation.

Unknown Lactarius + jack pine ectomycorrhizae were those with (1) smooth mantle with none or very little extramatrical mycelium, and (2) mantle cells forming a textura epidermoidea. This concept may include fungi other than those in the genus Lactarius but at least L. rufus + sitka spruce has the same hyphal arrangement (Alexander, 1981).

As a further aid in identification, distinctive ectomycorrhizal types were washed, surface sterilized in 30% H_2O_2 for 10 sec and 20 tips of each type plated on benomyl-MMN agar. Cultures resulting from these isolations were compared to stock cultures obtained from fruit bodies.

Specimens and cultures of a majority of the symbionts are on deposit with the Biosystematics Research Institute (DAOM), Agriculture Canada, Ottawa.

5.2.3 Results

About 50 species of confirmed or suspected ectomycorrhizal fungal symbionts of jack pine were collected at the Mildred Lake site (Table 27). Although there were a large number of ectomycorrhizal fungi fruiting on the site, specimens were sparce and widely scattered for all but a few species. In the cutline the most common species were Thelephora terrestris, Laccaria proxima, Hebeloma sp., and Scleroderma macrorhizon. None of these were observed in the

Table 27. Presumed ectomycorrhizal symbionts of jack pine collected in a mature jack pine-lichen stand and an adjacent cutline at Mildred Lake in northeastern Alberta (4 years observations).

Fungal Species	Number of years observed in: Mature Stand	Cutline	Growth in Culture	Mycorrhizal Status confirmed in pure culture by:[3]
Armillaria ponderosa (Pk.) Sacc.	1	0	+	NT
Astraeus hygrometricus (Pers.) Morgan	1	4	NA[2]	RMD
Chroogomphus rutilus (Schaeff.: Fr.) O.K. Miller	1	0	NA	NT
Coltrichia perennis (Fr.) Murrill	0	1	+	RMD
Cortinarius semiganguineus Fr.	3	0	-	NT
Cortinarius spp. (about 10 species)	4	3	-	NT
Entoloma sp. 2585[1]	0	1	NA	NT
Entoloma sp. 3012	1	0	+	NT
Hebeloma sp. 2657	0	3	+	RMD
Hydnellum zonatum (Fr.) Karst.	3	0	+	RMD
Hydnum sp. 3009	1	0	+	RMD
Hygrophorus eburneus (Fr.) Fr.	3	0	-	NT
Hygrophorus sp.	4	0	-	NT
Inocybe sp.	0	3	-	NT
Laccaria proxima Boudier	0	3	+	RMD
Lactarius affinus Pk. var. *affinus*	2	0	+	NT
L. paradoxus Beardslee & Burlingham	0	2	+	RMD
L. deliciosus (Fr.) S.F. Gray	1	0	+	JWK
L. resimus (Fr.) Fr.	1	0	+	NT
L. rufus (Scop.: Fr.) Fr.	2	0	+	IJA
L. villosus Clements	0	1	+	NT
Leccinum aurantiacum (Bull.) S.F. Gray	2	0	+	NT
Leptonia sp. 3021	1	0	+	NT
Neolecta vitellina (Bres.) Korf & Rogers	2	0	-	NT
Ramaria sp. 2954	1	0	NA	NT

Table 27. Presumed ectomycorrhizal symbionts of jack pine collected in a mature jack pine-lichen stand and an adjacent cutline at Mildred Lake in northeastern Alberta (4 years observations). (continued)

Fungal Species	Number of years observed in: Mature Stand	Cutline	Growth in Culture	Mycorrhizal Status confirmed in pure culture by:[3]
Rhizopogon rubescens Tul. var. *rubescens*	0	1	+	RMD
Russula spp. (about four species)	4	4	-	NT
Scleroderma macrorhizaon Chev.	0	3	+	RMD
Sistotrema confluens Fr.	2	0	+	RMD
Suillus albidipes (Pk.) Sing.	3	1	+	RMD
S. brevipes (Pk.) Kuntze	2	0	+	LFG
S. granulatus (Fr.) Kuntze	1	0	+	JWR
S. tomentosus (Kauff.) Sing. Snell & Dick	3	0	+	RMD
Thelephora terrestris (Ehrh.) Fr.	1	3	+	RMD
Tricholoma flavovirens (Pers. ex Fr.) Lundell	4	1	+	RMD
T. pessundatum var. *montanum* (Fr.) Gillet	2	0	+	RMD
T. saponoceum (Fr.) Staude	2	0	-	BN
T. zelleri (Smith & Stuntz) Ovrebo	1	0	+	RMD
T. spp. (about 5 species)	4	2	NA	NT

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[1] Numbers refer to voucher specimen numbers.
[2] NA = Culturing not attempted.
[3] NT = Not Tested; RMD = R.M. Danielson; LFG = L.F. Grand (1968); JWR = J.W. Riffle (1973); IJA = I.J. Alexander (1981); BN = B. Norkrans (1949).

adjacent mature stand except for one record of *T. terrestris*. The most common species in the mature stand were *Hygrophorus subalpinus*, *Lactarius resimus* and *Tricholoma flavovirens*. At a generic level, the most common symbionts fruiting in the jack pine stand were *Cortinarius*, *Hygrophorus*, *Lactarius*, *Suillus* and *Tricholoma*. The only hypogeous species found was *Rhizopogon rubescens* and all the suspected symbionts were Basidiomycetes except *Neolecta vitellina*, an inoperaculate Ascomycete.

Fruit bodies of fungi presumed to be saprophytes were also collected and cultured. These included two species of *Clitocybe*, three species of *Collybia*, *Gymnopilus* sp., *Hygrophoropsis aurantiacus* (Fr.) Schroeter, *Hygrophorus conicus* (Fr.) Fr. *Lyophyllum* sp., *Melanoleuca* sp., *Pholiota* sp., *Cystoderma granulosum* (Batsch ex Fr.) Fayod, *Lycoperdon marginatum* Vitt. and *L. pusillum* Pers.

Ectomycorrhizae recovered from cores taken in the cutline were not quantified in that most of the roots were shriveled and inactive. A small percentage of the root tips were infected with *Cenococcum geophilum* but no *Tricholoma flavovirens* or *Astraeus hygrometricus* types were observed. The most common type present was a coralloid form which resumbled ectomycorrhizae formed by *Lactarius* species. Ornamented hyphae resumbling those of the E-strain were also observed but no ectendomycorrhizae were found.

At both sampling dates, a majority of the ectomycorrhizae in the mature stand were active and were quantified. There was a large amount of variation in the total tips per liter soil and matched T-tests did not detect significant differences between the June and September samples (Table 28). *Cenococcum geophilum* exhibited the least variation and occurred in 27 of the 30 samples. It occurred at fairly low levels throughout the stand, exceeding 20% of the total ectomycorrhizae in only four samples. In contrast, *Tricholoma flavovirens* which occurred in 16 samples, exceeded 20% in 9 samples. Overall, about 40% of the ectomycorrhizae were formed by *Lactarius* spp. and *T. flavovirens*. *Suillus* spp., which fruited fairly commonly in the area appeared to form an insignificant portion of the ectomycorrhizae.

Table 28. Total number of active ectomycorrhizal root tips and frequency of occurrence of specific ectomycorrhizal fungi in the top 10 cm of mineral soil in an undisturbed jack pine-lichen woodland at Mildred Lake in northeastern Alberta.

Sampling Date	Total tips/L soil	% of ectomycorrhizal tips infected with:				
		Tricholoma Flavovirens	Cenococcum geophilum	Lactarius paradoxus	Lactarius spp.	Suillus spp.
June Mean ± SD[1]	4020 ± 2324	30.7 ± 33.7	8.5 ± 11.5	12.9 ± 20.4	19.5 ± 34.0	.4 ± .9
June Range	1513 - 10455	0 - 86.2	.2 - 36.8	0 - 62.6	0 - 90.8	0 - 2.1
Sept Mean ± SD	3204 ± 1689	11.2 ± 18.3	10.0 ± 13.4	3.0 ± 4.7	26.5 ± 38	1.8 ± 1.2
Sept Range	835 - 5790	0 - 53.4	0 - 44.9	0 - 14.5	0 - 96.5	0 - 3.6

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[1] No significant differences due to time in any category as determined by matched pair t-tests ($p = .05$).

With the exception of Cenococcum geophilum and a darkly pigmented Basidiomycete, Tomentella sp. (R-1851), which formed 25 of the 27,600 ectomycorrhizae examined, all of the ectomycorrhizae were formed by species with hyaline or very lightly pigmented hyphae. The Tomentella isolate was identified when it fruited on MMN agar after 3 months incubation. The Basidiospores were ornamented like species of Thelephora (Corner, 1968) and the tomentelloid fungi (Larsen, 1968) and since the fruit bodies were strictly resupinate, it is referred to the genus Tomentella.

5.4 DISCUSSION

In contrast to most other studies of ectomycorrhizae in natural forests, a large portion of the ectomycorrhizal fungi could be associated with fungi fruiting on the site. Past studies have indicated that few ectomycorrhizae were formed by the agarics and boletes that fruit commonly in forests (Lamb, 1979; Lamb and Richards, 1970; Riffle, 1973; Zak and Bryan, 1963). The current study as well as that of Chu-Chow (1979) in which a large percentage of the ectomycorrhizae of Pinus radiata D. Don were shown to be formed by Rhizopogon spp., suggests that in some forests, agarics and their allies are important ectomycorrhizal symbionts. The failure to identify additional ectomycorrhizae may be due to restricted spacial distribution, i.e. widely spaced clumps of many species which would necessitate extremely intensive sampling for detection. In addition, many species are extremely variable in culture, thus making match-ups between ectomycorrhizal isolates and fruit body isolates very difficult unless the full range of variability has been accounted for. It is clear that large numbers of isolations must be made from fruit bodies and monoxenic syntheses performed in order to identify the important symbionts.

Although the soil at the Mildred Lake site was subject to drought, there was no significant change in the quantity of ectomycorrhizae between early summer and fall. In drought-prone conifer forests in Montana, very substantial seasonal decreases occur in the numbers of active ectomycorrhizae (Harvey et al., 1978). The density of ectomycorrhizae in the Mildred Lake soil was similar to that found by Mikola and Laiho (1962), less than found in a loblolly pine plantation (Menge et al., 1977) and considerably greater than found by Harvey et al. (1978). Harvey et al (1979) suggested that the number of ectomycorrhizae increased with increasing site productivity.

Two instances of specialized fruiting were noted in the mature stand. Cortinarius semisanguineus fruited most frequently on jack pine logs which were at the brown cubical rot stage of decay. It is very likely that decayed wood served as a substrate for jack pine + C. semisanguineus ectomycorrhizae. In forest soils in Montana, decayed wood has been shown to be an important site of ectomycorrhizae formation as well as a source of moisture for plant growth during dry periods (Harvey et al., 1979). Thus, C. semisanguineus may be an important symbiont in mature jack pine stands which contain substantial amounts of decaying wood.

The second instance of specialized fruiting involved the apparent stimulation of fruiting of certain symbionts by disturbance. Sistotrema confluens fruited exclusively on the sides of soil core holes both 3 and 15 months after the cores had been removed. Also observed fruiting in the core holes were Hydnellum zonatum, Entoloma sp., Hygrophorus sp. and an unknown agaric. Intentional disturbance (e.g. trenching) might be used to induce fruiting of other species.

The inclusion of Neolecta vitellina as a potential symbiont is provisional and based on the field observations of Ogawa (1977) who considered it to be ectomycorrhizal with Pinus densiflora. However, Redhead (1979) observed it to be parasitic on ectomycorrhizal roots. Attempts to culture it from ascospores and pieces of the fruit bodies during this study failed. It is worthy of additional study in that it and N. irregularis (Pk.) Korf & Rogers are widespread in Canadian coniferous forests (Redhead, 1977).

Hydnums were only found in mature stands and have not been reported by others to occur in disturbed areas (e.g. Hintikka and Naykki, 1967). This probably is due to their very slow rates of growth (Hintikka and Naykki, 1967) and subsequent high stability as indicated by species fruiting in the same spot for decades (Harrison, 1971). It is likely that all the hydnums that fruit on soil are ectomycorrhizal even though they have never been considered in mycorrhizal research programs. Although species such as Hydnellum zonatum are very slow growing in culture, they might be useful in inoculation programs due to their apparent stability and long lived nature. Trials are required to test some hydnums against some of the pioneer (and perhaps more ephemeral) species that are currently being used in nursery inoculation research.

The most likely traditional candidates for inoculation trials are those found in the cutline. Species of Laccaria and Hebeloma, very similar to those found at Mildred Lake, are promising candidates for seedling inoculations (J. Trappe, pers. comm.). Observations reported here indicate that species in these genera are successful symbionts in northern Alberta.

Pisolithus tinctorius is not known to occur in Alberta and is rare in other parts of Canada (Malloch and Kuja, 1979). This indicates that it is ill-adapted to Canadian conditions and despite its success in the southern U.S., other fungi should be considered for inoculation in Canada. In addition to Hebeloma sp. and Laccaria proxima, Astraeus hygrometricus should be considered. The fruit body development of A. hydrometricus shows a clear adaptation to droughty, sandy soils and it is likely that the ectomycorrhizae formed by A. hygrometricus would show similar adaptations. Although more slow growing than P. tinctorius, it has several similarities with P. tinctorius which should justify its inclusion in any inoculation program with jack pine. Both Astraeus hygrometricus and Pisolithus tinctorius are Gasteromycetes and have pigmented hyphae and form mycelial strands. They, along with species of Scleroderma, especially S. macrorhizon, occur in sand blows in Wisconsin (Phelps, 1973), mine wastes in Pennsylvania (Schramm, 1966) and in the mountains of Italy (Pacioni, 1978). This Astraeus-Scleroderma-Pisolithus association suggests that either Scleroderma or Astraeus might be equally as useful a symbiont as P. tinctorius. Scleroderma macrorhizon forms ectomycorrhizae with jack pine but is slow-growing and difficult to maintain in culture (R. Danielson, unpublished data). Thus, of the Gasteromycete species found in droughty areas, A. hygrometricus appears to be the best choice for inoculum testing. It has been used successfully in a preliminary greenhouse inoculation trial (R. Danielson, unpublished data). In that Pisolithus tinctorius has been shown to possess considerable variation in growth rates and effectiveness in forming mycorrhize (Molina, 1979), additional isolates of Astraeus hygrometricus should be sought and cultured.

6. GENERAL CONCLUSIONS

1) The use of complimentary techniques (direct counts and isolations) allowed nearly all the fungal components of the ectomycorrhizae in the tank study to be tentatively identified and quantified. Mycological methodology is required to monitor ectomycorrhizal populations in disturbed areas.

2) There were only a small number of species of mycorrhizal fungi in the amended spoils and these were almost entirely Aphyllophorales and Ascomycetes. Hebeloma sp., Inocybe sp., and Suillus spp. were the only Agaricales detected over the course of 5 years. The most abundant species all had brown pigmented hyphae unlike the species found in undisturbed jack pine or white spruce stands.

3) The initial mycorrhizal associations formed were relatively stable, persisting for at least several years. Thus, mycorrhizal management must be fully considered prior to spoil treatments in order to establish symbioses and to ensure that effective symbionts are introduced.

4) The spread of ectomycorrhizal fungi from plant to plant was relatively slow. Thus if the entire plant population is to benefit from the symbiosis during the period of plant establishment, the inoculum must be abundant and dispersed over the entire spoil area or present on each individual when planted.

5) The effects of amending spoils and tailings on ectomycorrhizal associations cannot be predicted unless specific host-symbiont combinations have been studied previously. For example, sewage sludge additions inhibited the formation of some symbioses whilst stimulating the formation of others.

6) The peat amendment was an important source of inoculum, introducing ectomycorrhizal fungi that persisted on the root systems for long periods.

7) Most ectomycorrhizae in the peat differed morphologically from those found in undisturbed sites. Notably, ectomycorrhizal fungi from peat lacked mycelial strands and large amounts of external mycelium, features which may be important in water conduction and uptake as well as nutrient conservation in coarse textured, droughty spoils.

8) A large portion of the ectomycorrhizae in the native jack pine stand were apparently formed by agarics, especially _Tricholoma_ spp. and _Lactarius_ spp. Hypogeous fungi and Ascomycetes (except _Cenococcum geophilum_) were rare.

9) Most ectomycorrhizal symbionts fruiting in the cutline adjacent to the jack pine stand were much less frequent in the mature stand. This suggests that most species present in mature forests are ill-adapted to conditions existing in barren disturbed soils.

10) The corticoid fungus, _Amphinema byssoides_, was found to be a widespread symbiont of pine and spruce, and displayed a very wide ecological amplitude.

11) A species of _Tomentella_, a genus previously thought to contain only wood decay fungi, was found in the peat and formed ectomycorrhizae with spruce, pine, bearberry and willow.

7. RECOMMENDATIONS FOR FUTURE RESEARCH

1) The jack pine, white spruce, and willow should be sampled in the sixth growing season to determine the persistence of _Thelephora terrestris_, the E-strain, _Amphinema byssoides_ and _Tomentella_ sp. in the two spoils.

2) Detailed studies should be conducted on species that hold promise for use in greenhouse ectomycorrhizal inoculation trials as well as those species that are likely to be encountered in reclamation efforts. These would include *Amphinema byssoides*, *Thelephora terrestris*, the E-strain, *Laccaria proxima*, *Astraeus hygrometricus*, *Cenococcum geophilum*, *Tricholoma* species and *Sphaerosporella brunnea*. Host specificity, efficiency, ectotypic variability and inoculation difficulties should be determined.

3) The persistence of the species named in (2) should be determined in field situations on amended spoil or tailings materials.

4) The field testing of a complete range of ectomycorrhizal symbionts is currently impossible due to the inability to establish many mycorrhizal combinations using current techniques for rearing container-grown seedlings. Intensive investigations should be made of the inoculation process of container-grown seedlings with regard to fertilizer regimes, planting mixture composition, and the planting mixture microflora.

5) As drought would be expected to be a prime stress factor on oil sand tailings, morphological, cultural and taxonomic factors should be evaluated in order to permit the selection of fungi that are adapted to low moisture conditions.

6) A survey should be conducted of nursery stock in Alberta in order to determine the ectomycorrhizal status of the roots and the potential for incorporating inoculation procedurers into the production of seedlings.

7) Additional stands of white spruce and jack pine should be studied with regard to mycorrhizal associations. Information is required on the general abundance of specific fungi for each forest type.

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9. APPENDIX 1: EVALUATIONS OF ECTOMYCORRHIZAL ROOT SYSTEMS WITH EMPHASIS ON JACK PINE.

9.1 INTRODUCTION

The purpose of this Appendix section is to present the routine techniques used in evaluating the ectomycorrhizal status of root systems. The techniques have been used primarily with white spruce and jack pine but illustrate the background information required and methods that can be applied in studies of these as well as other host species. It is felt that quantification of total ectomycorrhizal infection alone is insufficient in field studies of ectomycorrhizae and more effort must be placed on identifying the symbionts, i.e. symbiont + host relationships. This is a difficult task, but without it the effects of environmental changes, e.g. land clearance, mining, silvicultural practices and toxic chemical inputs, cannot be properly evaluated. General aspects of ectomycorrhizal quantifications have been given by Grand and Harvey (1982).

9.2 GENERAL OBJECTIVES OF ECTOMYCORRHIZAL ASSESSMENTS

1. To determine the intensity of ectomycorrhizal infection as indicated by the percentage of short roots converted to ectomycorrhizae. Short roots of a single plant may become infected by soil-borne propagules, external hyphal growth from an ectomycorrhiza to adjacent noninfected short roots or by internal spread of the fungal symbiont within the cortex of long lateral roots. Thus, percent ectromycorrhizal infection in itself cannot be considered to be a measure of soil infectivity or inoculum potential (sensu Garrett, 1970) of the fungal symbionts. Once infection has occurred the inoculum potential of the fungus will be increased dramtically and soil infectivity over-estimated by short root and ectomycorrhizae counts.

2. Determination of the species of fungi forming ectomycorrhizae. This is far more difficult than simply determining the

level of total ectomycorrhizal infection and usually requires additional techniques. Few fungi can presently be identified from observations of the ectomycorrhizae although repeated reports on the occurrence of Cenococcum geophilum suggests otherwise. However, C. geophilum is one of the few exceptions and few mycorrhizal associations have been studied in sufficient detail to allow identification of the fungal symbiont. Standard mycological techniques, especially for obtaining fungi in pure culture, are required to identify and quantify specific fungal symbionts.

9.3 SHORT ROOTS VERSUS LONG LATERALS (SENSU SUTTON, 1980).

In order to quantify ectomycorrhizal infections it is first necessary to recognize the basic unit of quantifying ectomycorrhizal infection, the short root. Short roots, or feeder roots as they are referred to by some authors, are all considered to be susceptible to infection by ectomycorrhizal fungi whereas long laterals are not susceptible or the hyphae are limited to intercellular spaces of the cortex. If young lateral roots are included in counts, the degree of infection may be underestimated and the total number of roots involved in nutrient and water uptake overestimated. These two functionally different types of roots of pine are distinguished by the following features.

Short Roots	Long Lateral Roots
1. Root apcies rounded with a small, sharply delimited meristermatic zone which is lens-shaped.	1. Root apices acute, strips of cortical cells are frequently seen sloughing off from the tip. The meristem is paraboloid, longer than in short roots.
2. Branching, if it occurs, is bifurcate or dichotomous at an acute angle.	2. Branching which produces either short roots or a higher degree of long laterals, is racemose at right angles to the mother root.

3. Length is usually less than 5 mm (rarely 10 mm), i.e. of limited extent.	3. Length is indeterminate.
4. Root diameter is thin (less than 0.5 of the mother root diameter) unless a thick fungal mantle is present.	4. Usually much thicker than short roots with diameter equal to or greater than 0.5 the diameter of the mother root.
5. Apices may be covered with a fungal mantle.	5. Apices not covered with a mantle of hyphae.
6. Root hairs may be present on uninfected short roots but are rarely present on infected short roots.	6. Root hairs common even on old laterals.
7. The root diameter does not vary greatly with changes in nutrient levels.	7. Under high nutrient levels the apical 2-3 cm may be enlarged 2-3 times the diameter of the older root.

A third type of root is found on pine species and termed subordinate mother roots by Wilcox (1968). These roots appear intermediate between short roots and long laterals and can either be grouped with short roots due to their susceptibility to infection by ectomycorrhizal fungi and limited potential for growth or counted separately. They constitute a minor part of the root system of pines but can cause confusion during ectomycorrhizal evaluations. These roots are characterized by the following features:

1. Apices rounded similar to short root tips but capable of obtaining lengths of 2-3 cm.
2. Branching is racemose and at right angles but infrequent.

3. Size is between long laterals and short roots.
4. The fungal mantle may cover the apical meristem unlike long laterals.

9.4 PREPARATION FOR COUNTING AND FUNGAL ISOLATIONS

1. Samples of roots are collected, which may be in soil cores of known volume from the field or entire root systems of small seedlings from greenhouse evaluations.

2. Wash roots to remove soil or planting mixture or soil under running water using a 2 mm sieve to prevent losses of broken roots. Further cleaning with an artist's brush or fine forceps (Number 5 jeweler's forceps) and needles may be necessary to remove soil or roots of nontarget plants so the short roots can be observed. It is necessary to use a dissecting microscope during the fine cleaning so as few ectomycorrhizae as possible are broken off.

3. Once clean, place the root system in petri dishes, cover with water to suppress the growth of molds and store at 5^{o}C. If possible, the roots should be evaluated within several days following removal from the soil.

4. If a random sampling of the ectomycorrhizae is desired, the entire root sample should be cut into 2-3 cm long segments, each of which will have 10-20 short roots.

5. A preliminary examination of the samples is useful in determining if any distinctive types are present or if special procedures will be required.

9.5 GENERAL RULES FOR COUNTING SHORT ROOTS

The counting and determination of the ectomycorrhizal status is often complicated by the complexity of morphology, root activity and artifacts due to the extraction and cleaning processes. In order to make the evaluations objective, it is necessary to follow some rules in the counting process.

1. Only "active" short roots are counted and rated. This determination is subjective as dead or inactive roots are often very

difficult to distinguish from live, active roots. Simple criteria have been reported by Harvey et al. (1976). Active short roots are those that are turgid, with sound colours, with a hyaline meristem, and with a pale-coloured cortex and stele.

2. Broken roots are not counted or rated with regard to ectomycorrhizal infection status as only the missing tip may have been infected. This is especially important with species such as Populus, Salix, and other fine-rooted species in which only the final millimeter of a 10 mm short root is often ectomycorrhizal. If the total number of short roots is required, the scars and broken roots must be counted separately and added to the number of intact roots. In such cases, it can only be assumed that breakage occurs equally to ectomycorrhizal and nonmycorrhizal roots (an invalid assumption in many cases).

3. The minimum size of short root that can be evaluated for infection is one in which the short root meristem extends beyond the cortex of the mother root. For convenience and to make decisions objective, all short roots are counted and rated in which the ratio of length of short root to diameter of short root exceeds 1.5.

4. Regardless of the amount of branching, each short root is considered a single ectomycorrhizal infection point or unit as branching may be induced by the fungal symbiont. In addition, all root tips can be counted to obtain data on the degree of branching and total ectomycorrhizal development (excluding extramatrical hyphal development and long lateral root colonization).

5. If a dichotomous root has one tip infected and one uninfected it is rated as one infected short root with one additional uninfected tip.

6. If the stem or base is infected with the dichotomous portion uninfected it is rated as one infected short root plus one additional infected tip as it is assumed that the branching is symbiont induced.

7. If two fungi are infecting the same short root, only the species on the tip (the youngest part) is recorded. Additional notes may be made on the apparent succession of fungal symbionts in progress.

9.6 COUNTING PROCEDURES AND CONFIRMATION OF ECTOMYCORRHIZAL INFECTION.

Many of the techniques used in quantifying the ectomycorrhizal status of root systems are based on experiences with the genus Pinus which have some of the most morphologically distinct ectomycorrhizae. However, ectomycorrhizae formed with many other genera of plants are much less easily recognized and require more critical evaluations (Wilcox, 1982). Even with pines, it is often not possible to confirm infection by ectomycorrhizal fungi using low magnifications. Evaluations must balance the factors of adequate sample size (number of short roots counted) and time required for the evaluations using the low magnification, high magnification and sectioning techniques.

1. All intact, active short roots are examined with the aid of a dissecting microscope at 10-50X (12X is the most efficient magnification) with incident light and rated as ectomycorrhizal or uninfected. The root sample is placed in a rectangular dish and covered with water. A clear plastic dish (a flat plastic lid works well) is preferable as the background colour can be changed from white to black readily. In case of inconspicuous ectomycorrhizae, a black background is best as individual hyaline hyphae can be seen radiating from the root surface, a feature not discernible with a white background. The sample size (number of short roots) will depend on a variety of factors but 300 short roots per sample is adequate in most cases. This sample is drawn randomly from the roots that were previously cut into 2 to 3 cm segments. The criteria used to determine if short roots are ectomycorrhizal using low magnification techniques are as follows.

Ectomycorrhizal	Nonmycorrhizal
1. The most obvious indication of infection is when short roots are converted into coralloid structures by infection with genera such as *Suillus* and *Rhizopogon*. More common is simple dichotomous branching in species of *Pinus*.	1. Usually monpodial with occasional dichotomous branching. However container-grown pine seedlings may exhibit extensive dichotomous branching in the absence of ectomycorrhizal fungi.
2. The presence of extramatrical hyphae or mycelial strands on short roots often indicate infection.	2. Large amounts of hyphae are usually absent.
3. The surface may be very smooth and polished.	3. The surface is coarse and flaky due to the large size of the exposed cortical cells.
4. The cortical region is opaque due to Hartig net development.	4. The cortical region is transluscent and appears to "sparkle" internally as no hyphae are present to obstruct the view.
5. Root hairs are almost always absent from infected portions but they may be present on the base of a short root while the tip is infected.	5. If root hairs are present along the entire length of a short root, it is uninfected. Rarely, short roots may become infected after root hairs have developed.

6. Infection results in an increased short root diameter, often appearing clavate with swollen tips.	6. Uninfected short roots are slender, often constricted 2 to 3 times and taper gradually to the tip.
7. Colour is useful only when bright, white or pure black.	7. Colours are nondistinct and bland.

2. To verify infections in doubtful cases, which may be frequent, the most efficient and sensitive technique is to examine whole mounts of short roots using high magnification. Short roots are mounted in water (do not squash) and the upper surface is examined with a 40X brightfield objective. The mantle can then be observed in plan view and if the mantle is thin or absent, the Hartig net can be easily seen. The presence of either a mantle or Hartig net is the critical and confirming criterion of the ectomycorrhizal state. Very early stages of infection can be detected as well as the hyphal arrangement of the mantle using whole mounts.

3. Final verifications of infections can be made with hand-cut sections (using Gillette double-edge stainless steel razor blades and elder pith) mounted in 0.05% trypan blue in lactophenol. The stain will intensify in colour if heated briefly with an alcohol lamp. These sections should be sealed the following day with clear fingernail polish. The trypan blue will increase in intensity for about a week during storage after which the hyphal elements can be easily seen with brightfield optics. The disadvantage of sectioning is the time required to cut and mount sections and the possibility that light infections may be overlooked. Whole mounts circumvent both of these disadvantages.

9.7 ISOLATION OF FUNGAL SYMBIONTS FROM SPECIFIC TYPES OF MYCORRHIZAE.

This supplements ectomycorrhizal counts and permits the identification of many symbionts that cannot be recognized by ectomycorrhiza morphology. The isolation technique is limited in that (1) many ectomycorrhizal fungi will not grow in pure culture on agar, (2) it is necessary to match ectomycorrhizal isolates with cultures from fruit bodies, and (3) it is necessary to observe a large number of cultures to account for interspecific variability.

Two procedures are very useful in isolating fungal symbionts from ectomycorrhizae; surface sterilization and the use of selective media. The addition of the common fungicide benomyl to media largely eliminates hyphomycetes while allowing Basidiomycetes and Phycomycetes to grow. A medium containing benomyl is the medium of choice if the fungus is known to be a Basidiomycete but it cannot be used alone if Ascomycetous symbionts are present. A simple but very effective method of surface sterilizing mycorrhizae is to briefly dip the mycorrhizae in 95% ethanol, immerse in 30% H_2O_2 for 15 sec and rinse in ice cold sterile water for 30 min. A general procedure for isolating ectomycorrhizal fungal symbionts is as follows:

1. Select 2 to 3 cm long segments of lateral roots with the desired ectomycorrhizal type. Wash thoroughly for 30 to 60 min with cold tap water in a 50 mL beaker with 1 mm mesh mylon netting secured over the top of the beaker.

2. For fungal symbionts bearing clamps on the hyphae, either plate directly on benomyl-MMN (Appendix Table 1) if the mantle is thin or surface sterilize prior to plating if the mantle is thick. For Ascomycete symbionts (identified by the presence of Woronin bodies associated with the septa), surface sterilize and plate on MMN+ (Appendix Table 1). For symbionts of unknown affinities surface sterilize and plate on both benomyl-MMN and MMN+. Ten tips, 1 to 2 mm long, are the minimum number plated for each type, five per plate.

Appendix Table 1. Media used for the isolation of mycorrhizal symbionts (MMN modified from Marx, 1969).

A. Modified Melin-Norkrans (MMN) plus antibiotics (MMN+)

$(NH_4)_2HPO_4$	0.25 g
$CaCl_2$	0.05 g
NaCl	0.025 g
KH_2PO_4	0.50 g
$MgSO_4 \cdot H_2O$	0.15 g
Sequestrene iron	0.048 g (10 mL stock solution[1])
Thiamine HCl	100 g (1 mL stock solution[2])
Malt extract	3 g
Dextrose	10 g
Agar	15 g
Water	1000 mL
Streptomycin	100 mg[3]
Chlorotetracycline	50 mg[3]

[1] Stock solution of 480 mg Sequestrene in 100 mL water; Green Cross brand, 10% Fe

[2] Stock solution 10 mg Thiamine HCl in 100 mL water

[3] Add the two antibiotics directly to 10 mL sterile water, dissolve and add to molten media just prior to pouring plates.

B. Benomyl-MMN Agar

Add 5 mL of stock solution of Benomyl to MMN+ just prior to pouring plates. Stock solution contains 0.2 g Benomyl in 100 mL acetone. Benomyl contains 50% active ingredients (Later Chemicals Ltd.).

3. Parafilm plates and check daily for at least one week for the growth of nonmycorrhizal fungi. If fast growing fungi such as Mucor grow from some of the mycorrhizal transfer the other tips to a fresh plate containing the same medium. The plates should be observed for 2 months before discarding as some ectomycorrhizal fungi are very slow growing.

4. Record recovery of probable symbionts, nontarget fungi and tips that remain free of fungal growth.

5. Attempt to match cultures with those obtained from fruit bodies of known ectomycorrhizal fungi.

9.8 PRESERVATION OF SPECIMENS OF MYCORRHIZAE.

Root and ectoectomycorrhizae can be preserved indefinitely in a weak solution of FAA if care is taken to prevent evaporation of the preservative. Weak FAA consists of 50% ethanol (90 mL), acetic acid (5 mL) and formaldehyde (5 mL) (Johansen, 1940).

9.9 KEY MORPHOLOGICAL CHARACTERISTICS OF "WILD" ECTOMYCORRHIZAE.

In a majority of instances, the taxonomic identity of the fungal symbionts occurring in natural populations cannot be determined. Regardless, attempts should be made to characterize the ectomycorrhizae. Dominik (1962) developed a wholly artificial scheme which utilized extramatrical hyphal and colour characteristics as major descriptors and other authors have simply used colour to subdivide ectomycorrhizae into groups. The latter approach is inadequate as, with a few exceptions, colour varies with age and environmental factors. A discussion of the classification of ectomycorrhizae and evaluations of the factors used in classifying them have been given by Zak (1973).

The characteristics selected here are stable and are indicators of taxonomic affinities or likely to be of importance in defining ecological preferences. Collection of such information over a wide range of soil types may result in defining the importance of certain morphological features under specific environmental conditions. The features to be noted are given in decreasing order of importance.

1. Ascomycete or Basidiomycete affinities. This can often be determined by observing whether clamps (Basidiomycetes) or Woronin bodies (Ascomycetes) are present at the septa. Dolipore septa cannot be seen with the light microscope except in very rare cases. An Ascomycete affinity is often suggested if the hyphae exceed 5 m in diameter and Woronin bodies should be searched for at young septa. For fungi that have simple septa and in which Woronin bodies cannot be seen, the growth on media containing benomyl can be used to demonstrate Basidiomycete affinities (Danielson, 1982).

2. Hyphal arrangement of the mantle. The arrangement of the hyphae of the mantle is one of the most stable and useful characteristics in identifying different types of mycorrhizae. The plan view (the view perpendicular to the surface) of the mantle is easily seen using whole mounts and 400-500X magnification with a high quality brightfield objective lens. The organization of the mantle hyphae can be categorized according to the textura types as illustrated by Korf (1973) and Eckblad (1968). Other terms have been used to describe hyphal or tissue arrangements but the textura type is the preferred system. Synenchyma, a term used by Chilvers (1968), and pseudoparenchyma refers to compact tissue with no interhyphal spaces in which the hyphal basis is difficult or impossible to discern. In the textura system, this would include textura prismatica, textura globulosa and textura angularis. Prosenchyma (Chilvers, 1968) and plectenchyma describe moderately compact tissue in which the hyphal elements are clearly distinguishable. This type is divided into four textura types including textura intricata with distinct interhyphal spaces and textura epidermoidea which lacks interhyphal spaces.

3. Presence of cystidia. Cystidia are sterile terminal cells whose morphology distinguishes them from nondifferentiated terminal hyphal cells. Ectomycorrhizae must be very carefully examined at 12-25X with a dissecting microscope to detect cystidia

as they are often less than 100 μm long. The determinant growth, which results in a fringe of cells projecting to a uniform length, distinguishes cystidia from young hyphae. The shape, size, wall thickness and septation are determined using whole mounts at high magnification. Care must be exercised when quantifying cystidial types as the cystidia are inducible by unknown factors and thus may be absent on some ectomycorrhizae formed by cystidial fungal species.

4. Presence of mycelial strands or rhizomorphs. If present, mycelial strands are usually obvious, although they may be lost during the root cleaning process. The degree of cell organization is an important factor in recognizing mycelial strands produced by different species.

5. Amount of extramatrical mycelium. This varies from apparently none to so copious that the roots cannot be seen. The actual amounts may be underestimated on cleaned root systems, thus this determination is somewhat subjective.

6. Hyphal pigmentation. The only pigments recorded here are brown wall pigments present in quantities sufficient to colour hyphae when examined at 500X. Other pigments, e.g. yellow or green, cannot be seen except in mass, i.e. on intact ectomycorrhizae.

7. Presence of materials on hyphal walls (excluding ornaments). Materials excreted and deposited or formed de nova on the outside of hyphal walls can be in the form of crystals, resinous exudates or encrustations. They may be so abundant that the hyphae are completely covered or occur as occasional patches. The presence of such materials appears to be consistent within a species and is particularly useful in recognizing *Rhizopogon*-*Suillus* ectomycorrhizae.

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Appendix Table 2. Effect of time of exposure to H_2O_2 and temperature of rinse water on recovery of the E-strain from jack pine mycorrhizae formed in 3 year old peat used to amend the subalpine spoil and oil sand tailings.

	Time of exposure to 30% H_2O_2 (Sec)			
	15	5	15	5
	Ice Water		Room Temp.	
Taxa	Percent Recovery			
E-strain[1]	88^a	66^{ab}	64^{ab}	52^b
Nonmycorrhizal	4^a	14^{ab}	18^{ab}	36^b

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[1] Data within each row analysed by 2-way ANOVA. Numbers superscripted with the same letter within each row not significantly different at p = .05 as determined by Scheffe multiple contrasts where F-test significant.

Appendix Table 3. Recovery of symbionts from mycorrhizae with and without surface sterilization and with rinsing in water at room temperature and in ice water.

	Not surface[1] sterilized		H_2O_2-RT		H_2O_2-Ice	
	Pine	Spruce	Pine	Spruce	Pine	Spruce
			Percent Recovery			
Thelephora	38	-	1	-	9	-
Unknown symbiont	2	-	1	-	0	-
Nonmycorrhizal	57	61	19	10	15	12
No Growth	3	0	74	0	76	4
E-strain - pure	-	11	-	39	-	56
E-strain - overgrown	-	28	-	51	-	28
E-strain total	-	39	-	90	-	84

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[1] Jack pine mycorrhizae from sewage treated spoil, plated on benomyl-MMN. Spruce from subalpine control, plated on MMN+. Both washed for 1 h prior to treatment.

Appendix Table 4. Efficiency of water and sucrose in the extraction of large chlamydospores from amended coal mine spoil.[1]

Sieve size and extractant	Percent of total chlamydospores extracted	
	E-strain	VA-type
53 μm water	4.3	54.6
125 μm water	1.0	11.5
53 μm sucrose	83.5	30.4
125 μm sucrose	11.2	3.5

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[1] In order to determine if E-strain chlamydospores could be extracted from the peat by the method used, chlamydospores collected from a synthesis jar were added to a sample of the Canmore peat. Using the standard settling time of 15 min, only 14% of the chlamydospores were extracted (Table 5). Extending the settling time to 75 min permitted extraction of an additional 18% of the total chlamydospores added. Resuspension and centrifugation of the sample resulted in the recovery of a similar number of chlamydospores. Overall, 64% of the added chalmydospores were recovered and 96% of these were on the 53 m sieve.

Appendix Table 5. Efficiency of recovery of 640 E-strain chlamydospores added to a single 10 g sample of the Canmore peat.

		Time elapsed after centrifugation (min)				Resuspended and Centrifuged	
Sieve size	Extractant	0	15	75	150	15	75
		Number of chlamydospores extracted					
53 µm	water	14	NT[1]	NT	NT	NT	NT
53 µm	sucrose	14	90	116	15	114	21
125 µm	sucrose	0	8	7	NT	3	6

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[1] NT - Not Tested.